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TB **09/701588**INTERNATIONAL APPLICATION
PCT/US99/13975INTERNATIONAL FILING DATE
21 June 1999 (21.06.99)PRIORITY DATE CLAIMED
20 June 1998 (20.06.98)TITLE OF INVENTION
ARTIFICIAL T HELPER CELL EPITOPES AS IMMUNE STIMULATORS FOR SYNTHETIC PEPTIDE IMMUNOGENSAPPLICANT(S) FOR DO/EO/US
WANG, Chang Yi

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. ☐ This is **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371 (b) and PCT Articles 22 and 39 (1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau)
 - b. ☐ has been transmitted by the International Bureau
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International application into English (35 U.S.C. 371(c)(2)). with oath
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) And Power of Attorney
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included.

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification
15. ☐ A change of power of attorney and/or address letter
16. ☒ Other items or Information
 - Copy of the Form PCT/RO/101Request
 - Copy of Specification
 - Verified Certification of Express Mailing Date (International Application) under 37 C.F.R. § 1.10(c)
 - Check in the amount of \$782.00 for the fee; (which includes \$ 40.00 for recordation of the assignment);
 - Statement under 37 CFR 1.821(f) sequence Listing; and
 - Return receipt postcard

U.S. APPLICATION NO (if known, see 37 CFR 1.51)		INTERNATIONAL APPLICATION NO		ATTORNEY'S DOCKET NO	
TBA 09/701588		PCT/US99/13975		1151-4158US1	
17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO.....\$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ...\$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2) paid to USPTO\$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33 (1) - (4)\$690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1) - (4).....\$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =					
Surcharge of \$130 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ --	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	48 - 20	28	X \$18 00	\$ 504.00	
Independent claims	2 - 3 =	0	X \$80 00	\$ --	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270 00	\$ 270.00	
TOTAL OF ABOVE CALCULATIONS =				\$ 1484.00	
Reduction of 1/2 for filing by small entity, if applicable. Applicant is a Small Entity and claims Small Entity Status.				\$ 742.00--	
SUBTOTAL =				\$ 742.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f))				\$ --	
TOTAL NATIONAL FEE =				\$ 742.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$ 40.00	
TOTAL FEES ENCLOSED				\$ 782.00	
				Amount to be refunded:	\$
				charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$782 00 to cover the above fees is enclosed b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-4500, ORDER NO.1151-4158US1. A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Morgan & Finnegan LLP 345 Park Avenue New York, NY 10154-0053 Telephone: 212-758-4800 Telecopier: 212-751-6849				SIGNATURE <u>Maria C.H. Lin</u> NAME Maria C.H. Lin 29.323 REGISTRATION NO.	

ARTIFICIAL T HELPER CELL EPITOPES AS IMMUNE
STIMULATORS FOR SYNTHETIC PEPTIDE IMMUNOGENS

FIELD OF THE INVENTION

This invention relates to a peptide immunogen
5 comprising a novel artificial T helper cell epitope (Th)
covalently linked to a desired target antigenic site
comprising B cell epitopes and optionally a general immune
stimulator sequence. The artificial Th epitope imparts to the
peptide immunogen the capability to induce strong T helper
10 cell-mediated immune responses and the production of
antibodies directed against the "target antigenic site." The
invention also provides for the advantageous replacement of
carrier proteins and pathogen-derived T helper cell sites in
established peptide immunogens by the novel artificial T
15 helper cell epitopes for improved immunogenicity.

Many rules have been developed for predicting the
amino acid sequences of T cell epitopes. However, because
there is no central unifying theory on how or what makes a
particular amino acid sequence useful as a T cell epitope, the
20 rules are empirical and are not universally applicable. Being
aware of these rules, the novel artificial T helper cell
epitopes of the present invention were developed,
nevertheless, by empirical research.

The peptide immunogens of the present invention are
25 useful for evoking antibody responses in an immunized host to
a desired target antigenic site, including sites taken from
pathogenic organisms, and sites taken from normally
immunosilent self-antigens and tumor-associated targets.
Accordingly, the peptides of the invention are useful in
30 diverse medical and veterinary applications, such as:
vaccines to provide protective immunity from infectious
disease; immunotherapies for treating disorders resulting from
malfunctioning normal physiological processes; immunotherapies
for treating cancer and as agents to intervene in normal
35 physiological processes to produce desirable results.

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For example, the novel artificial T helper cell epitopes of the present invention provide novel short peptide immunogens that elicit antibodies targeted to luteinizing hormone-releasing hormone (LHRH) and are useful for

5 contraception, control of hormone-dependent tumors, prevention of boar taint, and immunocastration. The novel artificial Th epitopes of the present invention have been found to provoke an immune response when combined with target B cell epitopes of various microorganisms/proteins/peptides. In addition to

10 LHRH, the artificial Th epitopes of the present invention have been found to be useful when linked to other target antigenic sites including somatostatin for growth promotion in farm animals; IgE for treatment of allergy; the CD4 receptor of T helper cells for treatment and prevention of HIV infection and

15 immune disorders; foot-and-mouth disease virus capsid protein for prevention of foot-and-mouth disease; HIV virion epitopes for prevention and treatment of HIV infection; the circumsporozoite antigen of *Plasmodium falciparum* for prevention and treatment of malaria; and Cholesteryl ester

20 transport protein (CETP) for prevention and treatment of arteriosclerosis.

BACKGROUND OF THE INVENTION

It is known that most antibody immune responses are cell-mediated, requiring cooperative interaction between

25 antigen-presenting cells, B cells (antibody-producing cells which also function as antigen-presenting cells), and T helper (Th) cells. Consequently, the elicitation of an effective antibody response requires that the B cells recognize the target antigenic site (B cell epitope) of a subject immunogen

30 and the T helper cells recognize a Th epitope. Generally, the T helper epitope on a subject immunogen is different from its B cell epitope(s) (Babbitt et al., *Nature*, 1985; 317: 359-361). The B cell epitope is a site on the desired target recognized by B cells which in response produce antibodies to

35 the desired target site. It is understood that the natural

conformation of the target determines the site to which the antibody directly binds. The T helper cell recognition of proteins is, however, much more complex and less well understood. (Cornette et al., in *Methods in Enzymology*, vol 5 178, Academic Press, 1989, pp 611-634).

Under present theories, evocation of a Th cell response requires the T helper cell receptor to recognize not the desired target but a complex on the membrane of the antigen-presenting cell formed between a processed peptide 10 fragment of the target protein and an associated class II major histocompatibility complex (MHC). Thus, peptide processing of the target protein and a three-way recognition is required for the T helper cell response. The three part complex is particularly difficult to define since the critical 15 MHC class II contact residues are variably positioned within different MHC binding peptides (Th epitopes) and these peptides are of variable lengths with different amino acid sequences (Rudensky et al., *Nature*, 1991; 353:622-627). Furthermore, the MHC class II molecules themselves are highly 20 diverse depending on the genetic make-up of the host. The immune responsiveness to a particular Th epitope is thus in part determined by the MHC genes of the host. In fact, it has been shown that certain peptides only bind to the products of particular class II MHC alleles. Thus, it is difficult to 25 identify promiscuous Th epitopes, i.e., those that are reactive across species and across individuals of a single species. It has been found that the reactivity of Th epitopes is different even among individuals of a population.

The multiple and varied factors for each of the 30 component steps of T cell recognition: the appropriate peptide processing by the antigen-processing cell, the presentation of the peptide by a genetically determined class II MHC molecule, and the recognition of the MHC molecule/peptide complex by the receptor on T helper cells have made it difficult to determine 35 the requirements for promiscuous Th epitopes that provide for broad responsiveness (Bianchi et al., EP 0427347; Sinigaglia

et al., chapter 6 in *Immunological Recognition of Peptides in Medicine and Biology*, ed., Zegers et al., CRC Press, 1995, pp 79-87).

It is clear that for the induction of antibodies, the immunogen must comprise both the B cell determinant and Th cell determinant(s). Commonly, to increase the immunogenicity of a target, the Th response is provided by coupling the target to a carrier protein. The disadvantages of this technique are many. It is difficult to manufacture well-defined, safe, and effective peptide-carrier protein conjugates for the following reasons:

1. Chemical coupling are random reactions introducing heterogeneity of size and composition, e.g., conjugation with glutataraldehyde (Borras-Cuesta et al., *Eur J Immunol*, 1987; 17: 1213-1215);

2. the carrier protein introduces a potential for undesirable immune responses such as allergic and autoimmune reactions (Bixler et al., WO 89/06974);

3. the large peptide-carrier protein elicits irrelevant immune responses predominantly misdirected to the carrier protein rather than the target site (Cease et al., *Proc Natl Acad Sci USA*, 1987; 84: 4249-4253); and

4. the carrier protein also introduces a potential for epitopic suppression in a host which had previously been immunized with an immunogen comprising the same carrier protein. When a host is subsequently immunized with another immunogen wherein the same carrier protein is coupled to a different hapten, the resultant immune response is enhanced for the carrier protein but inhibited for the hapten (Schutze et al., *J Immunol*, 1985; 135: 2319-2322).

To avoid these risks, it is desirable to replace the carrier proteins. T cell help may be supplied to a target antigen peptide by covalent binding to a well-characterized promiscuous Th determinant. Known promiscuous Th are derived from the potent pathogenic agents such as measles virus F

protein (Greenstein et al., *J Immunol*, 1992; 148: 3970-3977) and hepatitis B virus surface antigen (Partidos et al., *J Gen Virol* 1991; 72: 1293-1299). The present inventors have shown that many of the known promiscuous Th are effective in

5 potentiating a poorly immunogenic peptide, such as the decapeptide hormone luteinizing hormone-releasing hormone (LHRH) (US 5,759,551). Other chimeric peptides comprising known promiscuous Th epitopes with poorly immunogenic

10 synthetic peptides to generate potent immunogens have been developed (Borras-Cuesta et al., 1987). Well-designed promiscuous Th/B cell epitope chimeric peptides are capable of eliciting Th responses with resultant antibody responses targeted to the B cell site in most members of a genetically diverse population (US 5,759,551).

15 A review of the known promiscuous Th epitopes shows that they range in size from approximately 15 to 50 amino acid residues (US 5,759,551) and often share common structural features with specific landmark sequences. For example, a common feature is the presence of amphipathic helices. These

20 are alpha-helical structures with hydrophobic amino acid residues dominating one face of the helix and charged and polar residues dominating the surrounding faces (Cease et al., 1987). Known promiscuous Th epitopes also frequently contain additional primary amino acid patterns such as a charged

25 residue, -Gly-, followed by two to three hydrophobic residues, followed in turn by a charged or polar residue (Rothbard and Taylor, *EMBO J*, 1988; 7:93-101). Th epitopes with this pattern are called Rothbard sequences. It has also been found that promiscuous Th epitopes often obey the 1, 4, 5, 8 rule,

30 where a positively charged residue is followed by hydrophobic residues at the fourth, fifth and eighth positions, consistent with an amphipathic helix having positions 1, 4, 5 and 8 located on the same face. This pattern of hydrophobic and charged and polar amino acids may be repeated within a single

35 Th epitope (Partidos et al., *J Gen Virol*, 1991; 72:1293-99).

Most, if not all, of the known promiscuous T cell epitopes contain at least one of the periodicities described above.

Promiscuous Th epitopes derived from pathogens include the hepatitis B surface and core antigen helper T cell epitopes (HBsAg Th and HBc Th), the pertussis toxin helper T cell epitopes (PT Th), the tetanus toxin helper T cell epitopes (TT Th), the measles virus F protein helper T cell epitopes (MVF Th), the *Chlamydia trachomatis* major outer membrane protein helper T cell epitopes (CT Th), the diphtheria toxin helper T cell epitopes (DT Th), the *Plasmodium falciparum* circumsporozoite helper T cell epitopes (PF Th), the *Schistosoma mansoni* triose phosphate isomerase helper T cell epitopes (SM Th), and the *Escherichia coli* TraT helper T cell epitopes (TraT Th). The sequences of these pathogen-derived Th epitopes can be found in US 5,759,551 as SEQ ID NOS:2-9 and 42-52 therein, incorporated herein by reference; in Stagg et al., *Immunology*, 1993; 79:1-9; and in Ferrari et al., *J Clin Invest*, 1991; 88: 214-222, also incorporated by reference.

The use of such pathogen-derived sites for the immuno-potentiation of peptide B cell sites for application to LHRH has been described in US 5,759,551, for HIV in Greenstein et al. (1992), for malaria in EP 0 427,347, for rotavirus in Borrás-Cuesta et al. (1987), and for measles in Partidos et al. (1991).

Useful Th epitopes may also include combinatorial Th epitopes. In Wang et al. (WO 95/11998), a particular class of combinatorial Th epitopes, a "Structured Synthetic Antigen Library" (SSAL) was described. Th SSAL epitopes comprise a multitude of Th epitopes with amino acid sequences organized around a structural framework of invariant residues with substitutions at specific positions. The sequences of the SSAL are determined by retaining relatively invariant residues while varying other residues to provide recognition of the diverse MHC restriction elements. This may be accomplished by

aligning the primary amino acid sequence of a promiscuous Th, selecting and retaining as the skeletal framework the residues responsible for the unique structure of the Th peptide, and varying the remaining residues in accordance with known MHC restriction elements. Lists of the invariant and variable positions with the preferred amino acids of MHC restriction elements are available to obtain MHC-binding motifs. These may be consulted in designing SSAL Th epitopes (Meister et al., Vaccine, 1995; 13:581-591).

The members of the SSAL may be produced simultaneously in a single solid-phase peptide synthesis in tandem with the targeted B cell epitope and other sequences. The Th epitope library sequences are designed to maintain the structural motifs of a promiscuous Th epitope and at the same time, accommodate reactivity to a wider range of haplotypes. For example, the degenerate Th epitope "SSAL1 TH1" (WO 95/11998), was modeled after a promiscuous epitope taken from the F protein of the measles virus (Partidos et al., 1991). SSAL1 TH1 was designed to be used in tandem with a target antigen, LHRH. Like the measles epitope from which it was derived, SSAL1 TH1 was designed to follow the Rothbard sequence and the 1, 4, 5, 8 rules and is a mixture of four peptides:

25	1	5	10	15	
	Asp-Leu-Ser-Asp-Leu-Lys-Gly-Leu-Leu-Leu-His-Lys-Leu-Asp-Gly-Leu				(SEQ ID NO:2)
	Glu Ile	Glu Ile Arg	Ile Ile Ile	Arg Ile Glu	Ile
					(SEQ ID NO:3)
30	Val	Val	Val Val Val	Val	Val
					(SEQ ID NO:4)
	Phe	Phe	Phe Phe Phe	Phe	Phe
					(SEQ ID NO:5)

A charged residue Glu or Asp is added at position 1 to increase the charge surrounding the hydrophobic face of the Th. The hydrophobic face of the amphipathic helix is then maintained by hydrophobic residues at 2, 5, 8, 9, 10, 13 and 16. Positions at 2, 5, 8, 9, 10, and 13 are varied to provide a facade with the capability of binding to a wide range of MHC

restriction elements. The net effect of the SSAL feature is to enlarge the range of immune responsiveness of the artificial Th (WO 95/11998).

Other attempts have been made to design "idealized" artificial Th epitopes" incorporating all of the properties and features of known promiscuous Th epitopes. Several peptide immunogens comprising these artificial promiscuous Th epitopes, including those in the form of SSAL, have also been constructed. The artificial Th sites have been combined with peptide sequences taken from self-antigens and foreign antigens to provide enhanced antibody responses to site - specific targets (WO 95/11998; Alexander et al., *Immunity*, 1994, 1:751; Del Guerio et al., *Vaccine*, 1997, 15:441) that have been described as highly effective. Such peptide immunogens are preferred for providing effective and safe antibody responses, and for their immunopotency, arising from a broadly reactive responsiveness imparted by the idealized promiscuous Th sites described.

SUMMARY OF THE INVENTION

The present invention provides an immunogenic peptide composition comprising a promiscuous artificial T helper cell epitope linked to a synthetic peptide B cell epitope or "target antigenic site". The immunogenic peptide comprise an artificial T helper cell (Th) epitopes and a target antigenic site containing B cell epitopes and, optionally, a general immune stimulator sequence. The presence of an artificial Th epitope in the immunogenic peptide impart thereto a capability to induce a strong T helper cell-mediated immune response with the production of a high level of antibodies directed against the "target antigenic site." The present invention further provides for the advantageous replacement of carrier proteins and pathogen-derived T helper cell sites in established peptide immunogens with artificial T helper cell epitopes designed specifically to improve their immunogenicity. The novel short peptide

immunogens with the artificial Th epitopes of the present invention elicit a high level of antibodies targeted to luteinizing hormone-releasing hormone (LHRH) useful for contraception, the control of hormone-dependent tumors, the prevention of boar taint, and immunocastration.

The artificial Th epitopes were developed empirically, mindful of the known rules for predicting promiscuous T cell epitopes. In the absence of a unifying theory explaining the mechanism of Th epitopes, these "predictive" rules serve merely as guidelines for designing effective artificial Th epitopes. The artificial Th epitopes of the present invention have been found to be useful when linked to target antigenic sites and optionally with a immunostimulatory sequence. The immunogenic peptides of the present invention may be represented by the formulae:

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$$(A)_n-(\text{Target antigenic site})-(B)_o-(\text{Th})_m-X$$

or

$$(A)_n-(B)_o-(\text{Th})_m-(B)_o-(\text{Target antigenic site})-X$$

or

5
$$(A)_n-(\text{Th})_m-(B)_o-(\text{Target antigenic site})-X$$

or

$$(\text{Target antigenic site})-(B)_o-(\text{Th})_m-(A)_n-X$$

or

$$(\text{Th})_m-(B)_o-(\text{Target antigenic site})-(A)_n-X$$

10 wherein: A is an amino acid or a general immunostimulatory sequence, e.g., the invasin domain (Inv) (SEQ ID NO:78) where n is more than one, the individual A's may be the same or different;

B is selected from the group consisting of amino
15 acids, $-\text{NHCH}(X)\text{CH}_2\text{SCH}_2\text{CO}-$, $-\text{NHCH}(X)\text{CH}_2\text{SCH}_2\text{CO}(\square\text{N})\text{Lys}-$, $-\text{NHCH}(X)\text{CH}_2\text{S-succinimidyl}(\square\text{N})\text{Lys}-$, and $-\text{NHCH}(X)\text{CH}_2\text{S-}$ (succinimidyl)-;

Th is an artificial helper T cell epitope selected from the group consisting of SEQ ID NOS:6-22, 31-35 and 105
20 and an analog or segment thereof;

"Target antigenic site" is a desired B cell epitope, a peptide hapten, or an immunologically reactive analog thereof;

X is an aminoacid $\alpha\text{-COOH}$, $-\text{CONH}_2$;
25 n is from 1 to about 10;
m is from 1 to about 4; and
o is from 0 to about 10.

An example of a peptide hapten as a target antigenic site is LHRH (SEQ ID NO: 77).

30 The compositions of the present invention comprise peptides capable of evoking antibody responses in an immunized host to a desired target antigenic site. The target antigenic site may be derived from pathogenic organisms and normally immunosilent self-antigens and tumor-associated targets such
35 as LHRH.

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Accordingly, the compositions of the present invention are useful in many diverse medical and veterinary applications. These include vaccines to provide protective immunity from infectious disease, immunotherapies for the treatment of disorders resulting from the malfunction of normal physiological processes, immunotherapies for the treatment of cancer, and agents to desirably intervene in and modify normal physiological processes.

Some of the targets antigens which may be covalently linked to the Th epitopes of the present invention include: LHRH for contraception, the control of hormone dependent tumors and immunocastration; somatostatin for growth promotion in farm animals; IgE for treatment of allergy; the CD4 receptor of T helper cells for treatment and prevention of HIV infection and immune disorders; foot-and-mouth disease virus capsid protein as a vaccine for the prevention of foot-and-mouth disease; the CS antigen of Plasmodium for prevention of malaria; CETP for prevention and treatment of arteriosclerosis; and HIV virion epitopes for prevention and treatment of HIV infection.

DETAILED DESCRIPTION OF THE INVENTION

Idealized artificial Th epitopes have been provided.

These are modeled on two known natural Th epitopes and SSAL peptide prototypes, disclosed in WO 95/11998. The SSALS incorporate combinatorial MHC molecule binding motifs (Meister et al., 1995) intended to elicit broad immune responses among the members of a genetically diverse population. The SSAL peptide prototypes were designed based on the Th epitopes of the measles virus and hepatitis B virus antigens, modified by introducing multiple MHC-binding motifs. The design of the other Th epitopes were modeled after other known Th epitopes by simplifying, adding, and/or modifying, multiple MHC-binding motifs to produce a series of novel artificial Th epitopes. The newly adapted promiscuous artificial Th sites were

incorporated into synthetic peptide immunogens bearing a variety of target antigenic sites. The resulting chimeric peptides were able to stimulate effective antibody responses to the target antigenic sites.

5 The prototype artificial helper T cell (Th) epitope shown in Table 1a as "SSAL1 Th1", a mixture of four peptides (SEQ ID NOS:2, 3, 4, 5) is an idealized Th epitope modeled from a promiscuous Th epitope of the F protein of measles virus (Partidos et al. 1991). The model Th epitope, shown in
10 Table 1a as "MVF Th" (SEQ ID NO:1) corresponds to residues 288-302 of the measles virus F protein. MVF Th (SEQ ID NO:1) was modified to the SSAL1 Th1 prototype (SEQ ID NOS:2, 3, 4, 5) by adding a charged residue Glu/Asp at position 1 to increase the charge surrounding the hydrophobic face of the
15 epitope; adding or retaining a charged residues or Gly at positions 4, 6, 12 and 14; and adding or retaining a charged residue or Gly at positions 7 and 11 in accordance with the "Rothbard Rule". The hydrophobic face of the Th epitope comprise residues at positions 2, 5, 8, 9, 10, 13 and 16.
20 Hydrophobic residues commonly associated with promiscuous epitopes were substituted at these positions to provide the combinatorial Th SSAL epitopes, SSAL1 Th1 (SEQ ID NOS:2, 3, 4, 5). The hydrophobic residues conforming to the Rothbard sequence rule are shown in bold (Table 1a, SEQ ID NO:1).
25 Positions in the sequence obeying the 1, 4, 5, 8 rule are underlined. Another significant feature of the prototype SSAL1 Th1 (SEQ ID NOS:2, 3, 4, 5) is that positions 1 and 4 is imperfectly repeated as a palindrome on either side of position 9, to mimic an MHC-binding motif. This "1, 4, 9"
30 palindromic pattern of SSAL1 Th1 was further modified in SEQ ID NO:3 (Table 1a) to more closely reflect the sequence of the original MVF model Th (SEQ ID NOS:1). Also, the hydrophobicity of the SSAL1 Th1 prototype (SEQ ID NOS:2, 3, 4, 5) was modulated in SEQ ID NOS:6, 7, and 8 by the addition of
35 methionine residues at variable positions 1, 12, and 14. Experimental data shows that SEQ ID NOS:6, 7, 8 coupled to a

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target antigenic site enhanced the antibody response in the immunized animals to the target antigenic site.

SEQ ID NOS:6, 7, 8 was simplified to SEQ ID NOS:6, 9, 10 and 11 (Table 1a) to provide further immunogenic SSAL Th epitopes. SEQ ID NOS:6, 7, 8 was further simplified to SEQ ID NOS:6, 12-14 (Table 1a) to provide a series of single-sequence epitopes. SSAL Th SEQ ID NOS:4, 9, 10 and 11 and the single sequence Th epitopes SEQ ID NOS:6, 12-14, coupled to target antigenic sites also provided enhanced immunogenicity

It was found that the immunogenicity of SEQ ID NOS:6, 7, 8 may be improved by extending the N terminus with a non-polar and a polar uncharged amino acid, e.g., Ile and Ser, and extending the C terminus by a charged and hydrophobic amino acid, e.g., Lys and Phe. This is shown in Table 1a as SEQ ID NO:15, 16 and 17 from which simplified SSAL Th epitopes SEQ ID NOS:15 and 18, 105 and 19 were derived. Peptide immunogens comprising a target antigenic site and a Th epitope selected from SEQ ID NOS:15-17, 15 and 18, 105 and 19, and 123 and 124 displayed enhanced immunogenicity. Single-sequence peptides such as SEQ ID NOS:15, 20-22 were also synthesized and tested for immunogenicity in animals. These were also found to be effective Th epitopes.

The SSAL artificial helper epitope shown in Table 1b as "SSAL2 Th2" (SEQ ID NOS:26-30) was modeled after a promiscuous epitope from the hepatitis B virus surface antigen SEQ ID NO:23 corresponding to residues 19-33 of the hepatitis B surface antigen (HBsAg) (Greenstein et al. 1992). The pathogen-derived model Th, was modified to SEQ ID NO:24 by adding three Lysines to improve solubility in water; the C-terminal Asp was deleted in SEQ ID NO:25 to facilitate the synthesis of chimeric peptides wherein Gly-Gly was introduced as spacers. The SSAL2 Th2 (SEQ ID NOS:26-30) was further modified from SEQ ID NO:24 by varying the positively charged residues therein at positions 1, 2, 3 and 5 to vary the charge surrounding the hydrophobic face of the helical structure. A charged amino acid at variable position 3 also contributed a

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required residue to generate the idealized Th epitope, SSAL2 Th2 (SEQ ID NOS:26-30), which obeyed the 1, 4, 5, 8 rule (underlined residues). The hydrophobic face of the amphipathic helix consists of hydrophobic residues at positions 4, 6, 7, 10, 11, 13, 15 and 17 of SEQ ID NOS:26-30. The Rothbard sequence residues are shown in bold for prototype SSAL2 Th2 (SEQ ID NOS:26-30).

SEQ ID NOS:31-35 were simplified from the idealized SSAL2 Th2 prototype (SEQ ID NOS:26-30) as described above. For example, variable hydrophobic residues were replaced with single amino acids, such as Ile or Met (SEQ ID NOS:31-35). The hydrophobic Phe in position 4 was incorporated as a feature of SEQ ID NO:34 while deleting the three lysines. The deletion of the C-terminal Asp was incorporated as a feature of SEQ ID NOS:32, 34, and 35. Further modifications included the substitution of the C-termini by a common MHC-binding motif AxTxIL (Meister et al, 1995).

Each of the novel artificial Th epitopes, SEQ ID NOS:6, 12-19, 105, 123, 124 20-22 and 31-35 were coupled to a variety of target antigenic sites to provide peptide immunogens. The target antigenic sites include the peptide hormones, LHRH and somatostatin, B cell epitopes from immunoglobulin IgE, the T cell CD4 receptor, the VP1 capsid protein of foot-and-mouth disease virus; the CS antigen of Plasmodium falciparum; and cholesteryl ester transport protein (CETP); and B cell epitopes from HIV. The results show that effective anti-target site antibodies cross-reactive with a diverse group of self-antigens and foreign antigens were produced. Most important, the antibody responses were directed to the target antigenic sites and not to the novel Th epitopes. The results for the novel peptide immunogens for LHRH are shown in Tables 2 and 3. The immunogenicity results also show that the antibodies produced were effective against LHRH but not against the Th epitopes themselves. It is to be emphasized that LHRH is a target antigenic site devoid of T cell epitopes (Sad et al., *Immunology*, 1992; 76: 599-603 and

US 5,759,551). Thus, the novel artificial Th epitopes of the present invention represent a new class of promiscuous T helper epitopes.

The artificial Th epitopes of the present invention are contiguous sequences of amino acids (natural or non-natural amino acids) that comprise a class II MHC molecule binding site. They are sufficient to enhance or stimulate an antibody response to the target antigenic site. Since a Th epitope can consist of continuous or discontinuous amino acid segments, not every amino acid of the Th epitope is necessarily involved with MHC recognition. The Th epitopes of the invention further include immunologically functional homologues. Functional Th homologues include immune-enhancing homologues, crossreactive homologues and segments of any of these Th epitopes. Functional Th homologues further include conservative substitutions, additions, deletions and insertions of from one to about 10 amino acid residues and provide the Th-stimulating function of the Th epitope.

The promiscuous Th epitopes of the invention are covalently linked to the N- or C- terminus of the target antigenic site, to produce chimeric Th/B cell site peptide immunogens. The term "peptide immunogen" as used herein refers to molecules which comprise Th epitopes covalently linked to a target antigenic site, whether through conventional peptide bonds so as to form a single larger peptide, or through other forms of covalent linkage, such as a thioester. Accordingly, the Th epitopes (e.g., SEQ ID NOS:6, 12-19, 105, 123, 124, 20-22 and 31-35) are covalently attached to the target antigenic site (e.g., SEQ ID NO:77) either via chemical coupling or via direct synthesis. The Th epitopes may be attached directly to the target site or through a spacer, e.g., Gly-Gly or (□-N)Lys. In addition to physically separating the Th epitope from the B cell epitope (e.g., SEQ ID NOS:41-64, 71-76, 84-90, 92-94, 96-102, 103, 104, 106, 126-129, and 136-153), the spacer may disrupt any artifactual secondary structures created by the linking of the Th epitope

or its functional homologue with the target antigenic site and thereby eliminate any interference with the Th and/or B cell responses. A flexible hinge spacer that enhances separation of the Th and IgE domains can also be useful. Flexible hinge sequences are often proline rich. One particularly useful flexible hinge is provided by the sequence Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO:79) modeled from the flexible hinge region found in immunoglobulin heavy chains. Xaa therein is any amino acid, preferably aspartic acid. The conformational separation provided by the spacer (See SEQ ID NOS:80 and 82) permits more efficient interactions between the presented peptide immunogen and the appropriate Th cells and B cells. Thus the immune responses to the Th epitope is enhanced to provide improved immune reactivity.

The peptide conjugate immunogens of the invention optionally may also comprise a general immunostimulatory peptide sequence. For example, a domain of an invasin protein (Inv) from the bacteria *Yersinia* spp (Brett et al., *Eur J Immunol*, 1993, 23: 1608-1614). This immune stimulatory property results from the capability of this invasin domain to interact with the $\beta 1$ integrin molecules present on T cells, particularly activated immune or memory T cells. A preferred embodiment of the invasin domain (Inv) for linkage to a promiscuous Th epitope has been previously described in US 5,759,551 and is incorporated herein by reference. The said Inv domain has the sequence:

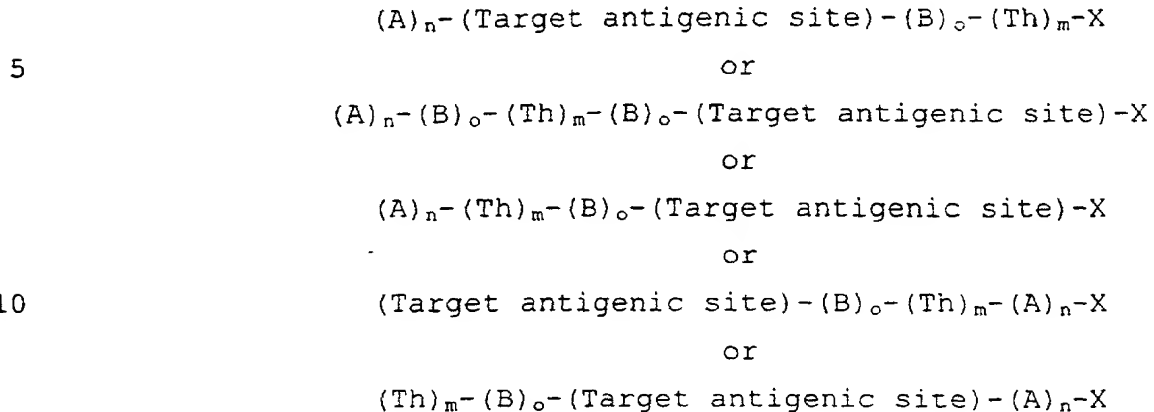
Thr-Ala-Lys-Ser-Lys-Lys-Phe-Pro-Ser-Tyr-Thr-Ala-Thr-Tyr-Gln-Phe

(SEQ ID NO:78)

or is an immune stimulatory homologue thereof from the corresponding region in another *Yersinia* species invasin protein. Such homologues thus may contain substitutions, deletions or insertions of amino acid residues to accommodate strain to strain variation, provided that the homologues retain immune stimulatory properties. The general immunostimulatory sequence may optionally be linked to the Th epitope with a spacer sequence.

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The peptide conjugates of this invention, i.e., peptide immunogens which comprise the described artificial Th epitopes, can be represented by the formulas:



wherein: A is optional and is an amino acid or a general immunostimulatory sequence, where $n > 1$, each A may be the same or different;

B is selected from the group consisting of amino acids, $-\text{NHCH}(X)\text{CH}_2\text{SCH}_2\text{CO}-$, $-\text{NHCH}(X)\text{CH}_2\text{SCH}_2\text{CO}(\square-\text{N})\text{Lys}-$, $-\text{NHCH}(X)\text{CH}_2\text{S-succinimidyl}(\square-\text{N})\text{Lys}-$, and $-\text{NHCH}(X)\text{CH}_2\text{S-succinimidyl}-$;

Th is an artificial helper T cell epitope (SEQ ID NOS:6, 12-19, 105, 20-22 and 31-35) or an immune enhancing homologue or segment thereof;

"Target antigenic site" is a desired B cell epitope or peptide hapten, or an analog thereof);

X is an amino acid α -COOH OR $-\text{CONH}_2$;

n is from 1 to about 10;

m is from 1 to about 4; and

o is from 0 to about 10.

The peptide immunogens of the present invention comprises from about 25 to about 100 amino acid residues, preferably from about 25 to about 80 amino acid residues.

When A is an amino acid, it can be any non-naturally occurring or any naturally occurring amino acid. Non-naturally occurring amino acids include, but are not limited

gto, D-amino acids, β -alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine, γ -amino butyric acid, homoserine, citrulline and the like. Naturally-occurring amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Moreover, when n is greater than one, and two or more of the A groups are amino acids, then each amino acid may be independently the same or different.

When A is an invasin domain sequence, it is preferably an immune stimulatory epitope from the invasin protein of an *Yersinia* species described here as SEQ ID NO:77.

In one embodiment where n is 3, each A is in turn an invasin sequence (Inv), glycine and glycine.

B is optional and is a spacer comprising one or more naturally occurring or non-naturally occurring amino acids. In $(B)_0$, where $0 > 1$, each B may be same or different. B may be Gly-Gly or Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO:79) or \square NLys or -NHCH(X)CH₂SCH₂CO-, -NHCH(X)CH₂SCH₂CO(\square NLys)-, -NHCH(X)CH₂S-succinimidyl- \square NLys-, and -NHCH(X)CH₂S-(succinimidyl)-.

Th is a promiscuous T helper cell epitope selected from the group SEQ ID NOS:6, 12-19, 105, 123, 124, 20-22 and 31-35 and homologues thereof.

The peptide immunogens of this invention, may be made by chemical methods well known to the ordinarily skilled artisan. See, for example, Fields et al., Chapter 3 in *Synthetic Peptides: A User's Guide*, ed. Grant, W.H. Freeman & Co., New York, NY, 1992, p.77. The peptides may be synthesized using the automated Merrifield solid phase peptide synthesis with either t-Boc or Fmoc to protect the α -NH₂ or side chain amino acids. Equipment for peptide synthesis are available commercially. One example is an Applied Biosystems Peptide Synthesizer Model 430A or 431.

After complete assembly of the desired peptide

immunogen, the resin is treated according to standard procedures to cleave the peptide from the resin and de-block the functional groups on the amino acid side chains. The free peptide is purified by HPLC and characterized biochemically, for example, by amino acid analysis, by sequencing, or by mass spectrometry. Methods of peptide purification and characterization are well known to one of ordinary skill in the art.

Other chemical means to generate peptide immunogens comprising the Th epitopes of the invention include the ligation of haloacetylated and cysteinylated peptides through the formation of a "thioether" linkage. For example, a cysteine can be added to the C terminus of a Th-containing peptide and the thiol group of cysteine may be used to form a covalent bond to an electrophilic group such as an N-chloroacetyl-modified or a maleimide-derivatized \square - or \square -NH₂ group of a lysine residue, which is in turn attached to the N-terminus of a target antigenic site peptide. In this manner, Th epitope/B cell site conjugates may be obtained.

The subject immunogen may also be polymerized. Polymerization can be accomplished for example by reaction between glutaraldehyde and the -NH₂ groups of the lysine residues using routine methodology. By another method, the linear Th/ B cell site immunogen can be polymerized or co-polymerized by utilization of an additional cysteine added to the N-terminus of the linear constructs. The thiol group of the N-terminal cysteine can be used for the formation of a "thioether" bond with haloacetyl-modified amino acid or a maleimide-derivatized \square - or \square -NH₂ group of a lysine residue that is attached to the N-terminus of a branched poly-lysyl core molecule (e.g., K₂K, K₄K₂K or K₈K₄K₂K). The subject immunogen may also be polymerized as a branched structure through synthesis of the desired peptide construct directly onto a branched poly-lysyl core resin (Wang, et al., *Science*, 1991; 254:285-288).

The longer synthetic peptide conjugates may

alternatively be synthesized by well known nucleic acid cloning techniques. Any standard manual on molecular cloning technology provides detailed protocols to produce peptides comprising the Th epitopes of the invention by expression of recombinant DNA and RNA. To construct a gene expressing a Th/target antigenic site peptide of this invention (e.g., SEQ ID NOS:36-64, 106, 71-76 and 80-82), the amino acid sequence is reverse translated into a nucleic acid sequence, preferably using optimized codons for the organism in which the gene will be expressed. Next, a gene encoding the peptide is made, typically by synthesizing overlapping oligonucleotides which encode the peptide and necessary regulatory elements. The synthetic gene is assembled and inserted into the desired expression vector. The synthetic nucleic acid sequences encompassed by this invention include those which encode the Th epitopes of the invention and peptides comprising those Th epitopes, the immunologically functional homologues thereof, and nucleic acid constructs characterized by changes in the non-coding sequences that do not alter the immunogenic properties of the peptide or Th epitope encoded thereby. The synthetic gene is inserted into a suitable cloning vector and recombinants are obtained and characterized. The Th epitopes and peptides comprising the Th epitopes are then expressed under conditions appropriate for the selected expression system and host. The Th epitope or peptide is purified and characterized by standard methods.

Peptide immunogens of the invention may be used alone or in combination to elicit antibody responses to Luteinizing Hormone Releasing Hormone. Luteinizing Hormone Releasing Hormone (LHRH) or Gonadotropin-releasing hormone (GnRH) is a master hormone for the regulation of sexual reproduction in both males and females. LHRH regulates the release of LH and FSH which in turn control spermatogenesis, ovulation and estrus, sexual development. LHRH ultimately controls the secretion of the male hormones androgen and testosterone, and the secretion of the female hormones,

estrogen and progesterone which themselves are essential for fertility in males and females, respectively. (*Basic and Clinical Endocrinology*, eds. FS Greenspan and JD Baxter, Appleton & Lange:Norwalk CT. 1994).

5 Active immunization against LHRH has long been known to exert multiple effects in males including decreased serum and pituitary LH and FSH, reduced serum testosterone, suppression of spermatogenesis and reversible atrophy of the gonads and accessory sex organs. (See, for example, Fraser et
10 al., *J. Endocrinol.*, 1974; 63:399-405; Giri et al., *Exp. Molec. Pathol.*, 1991; 54:255-264; Ladd et al., *J. Reprod. Immunol.*, 1989; 15:85-101; and references cited therein). Immunization against LHRH has been proven useful as a contraceptive in males and has potential as a treatment for
15 prostate cancer (Thau, *Scand J Immunol*, 1992; 36 Suppl 11:127-130; and US 5,759,551).

Immune intervention on the hypothalamo-pituitary gonadal axis by active immunization against LHRH can also be used to inhibit sexual hormones in females. Since LHRH
20 regulates the production of FSH by the anterior pituitary which in turn regulates the production of estrogen by the ovaries, blocking the action of LHRH is a therapy for sexual hormone-dependent diseases in women. For example, the ectopic development and maintenance of endometrial tissues outside the
25 uterine musculature is mediated by estrogen. Therefore, blocking the action of LHRH is useful as a treatment for endometriosis. Furthermore, by analogy to prostate cancer, estrogen-driven tumors of the breast should also be responsive to LHRH immunotherapy. Indeed, an anti-LHRH inducing vaccine
30 has been shown to effectively reduce serum levels of LH and FSH in women, an illustration of the potential of this method to effect contraception and treatment of hormone-dependent disorders (Gual et al., *Fertility and Sterility*, 1997; 67: 404-407).

35 In addition to providing treatment for a number of

important diseases and reversible infertility in both men and women, LHRH-based immunotherapy provides a means for reversible contraception in male and female animals (e.g. dogs, cats, horses and rabbits) as well as mitigating
5 undesirable androgen-driven behavior such as heat, territorial marking and aggression.

Lastly, immunological castration (e.g., antibody-based inhibition of LHRH action) has application in the livestock industry. Meat from male animals is not processed
10 into prime cuts because of the presence of an offensive aroma and taste, known as boar taint. Boar taint is conventionally eliminated by mechanical castration; however, castration of male food animals is no longer considered humane. Moreover, mechanical castration results in poorer growth performance and
15 lesions in body part, also referred to as carcass traits, in comparison to non-castrated males. Whereas, the growth performance and carcass traits of immunocastrated animals are less affected than those of castrated animals (Bonneau et al., *J Anim Sci*, 1994; 72: 14-20 and US 5,573,767). Therefore,
20 immunological castration is preferable to mechanical castration.

LHRH (or GnRH) is a self-molecule that must be linked to a Th component in order to generate anti-LHRH antibodies (Sad et al., *Immunology*, 1992; 76: 599-603).
25 Several such immunogenic forms of LHRH have been tested. For example, LHRH immunogens have been produced by conjugation to carrier proteins or linked by peptide synthesis to potent Th sites derived from pathogenic organisms (WO 94/07530, US 5,759,551, Sad et al., 1992). Improved LHRH peptide
30 immunogens comprising LHRH and artificial Th epitopes are exemplified in EXAMPLES 1-3.

This invention also provides for compositions comprising pharmaceutically acceptable delivery systems for the administration of the peptide immunogens. The
35 compositions comprise an immunologically effective amount of one or more of the peptide immunogens of this invention. When

so formulated, the compositions of the present invention comprising LHRH or a homologue thereof as target antigenic site, are used for treatment of prostate cancer, prevention of boar taint, immunocastration of animals, the treatment of
5 endometriosis, breast cancer and other gynecological cancers affected by the gonadal steroid hormones, and for contraception in males and females. The utility for peptides of the invention having target antigenic sites other than LHRH will vary in accordance with the specificity of the target
10 antigenic site.

The peptide immunogens of the invention can be formulated as immunogenic compositions using adjuvants, emulsifiers, pharmaceutically-acceptable carriers or other ingredients routinely provided in vaccine compositions.
15 Adjuvants or emulsifiers that can be used in this invention include alum, incomplete Freund's adjuvant (IFA), liposyn, saponin, squalene, L121, emulsigen, monophosphoryl lipid A (MPL), dimethyldioctadecylammonium bromide (DDA), QS21, and ISA 720, ISA 51, ISA 35 or ISA 206 as well as the other
20 efficacious adjuvants and emulsifiers. Such formulations are readily determined by one of ordinary skill in the art and also include formulations for immediate release and/or for sustained release. The present vaccines can be administered by any convenient route including subcutaneous, oral,
25 intramuscular, intraperitoneal, or other parenteral or enteral route. Similarly the immunogens can be administered in a single dose or multiple doses. Immunization schedules are readily determined by the ordinarily skilled artisan.

The composition of the instant invention contains an
30 effective amount of one or more of the peptide immunogens of the present invention and a pharmaceutically acceptable carrier. Such a composition in a suitable dosage unit form generally contains about 0.5µg to about 1 mg of the peptide immunogen per kg body weight. When delivered in multiple
35 doses, it may be conveniently divided into an appropriate amount per dose. For example, the dose, e.g. 0.2-2.5mg;

preferably 1 mg, may be administered by injection, preferably intramuscularly. This may be followed by repeat (booster) doses. Dosage will depend on the age, weight and general health of the subject as is well known in the vaccine and therapeutic arts.

Vaccines comprising mixtures of the subject peptide immunogens, particularly mixtures comprising Th sites derived from both MVF Th, i.e., SEQ ID NOS:6, 12-19, 105, 123, 124, 20-22, and HBsAg Th, i.e., SEQ ID NOS:31-35, may provide enhanced immunoefficacy in a broader population and thus provide an improved immune response to LHRH or other target antigenic site.

The immune response to Th/LHRH peptide conjugates or other Th/target antigenic site conjugates can be improved by delivery through entrapment in or on biodegradable microparticles of the type described by O'Hagan et al. (Vaccine, 1991; 9:768). The immunogens can be encapsulated with or without an adjuvant, and such microparticles can carry an immune stimulatory adjuvant. The microparticles can also be coadministered with the peptide immunogens to potentiate immune responses

As a specific example, the invention provides a method for inducing anti-LHRH antibody by administering pharmaceutical compositions comprising Th/LHRH peptide immunogens to a mammal for a time and under conditions to produce an infertile state in the mammal. As used herein an infertile state is that state which prevents conception. Infertility can be measured by methods known in the art, e.g. evaluation of spermatogenesis or ovulation, as well as by statistical modeling of experimental animal data. Other indicators of infertility in males includes reduction of serum testosterone to castration levels and involution of the testes. The appropriate dose of the composition is about 0.5µg to about 1mg of each peptide per kg body weight. This dosage may conveniently be divided into appropriate amounts

per dose when delivered in multiple doses.

Similarly, the LHRH embodiments of this invention relate to a method for treating androgen-dependent carcinoma by administering the subject peptide compositions to the
5 mammal for a time and under conditions to prevent further growth of the carcinoma. The appropriate unit dose is about 0.5 μ g to about 1mg of each peptide per kg body weight. This is conveniently divided into the appropriate amounts per application when administered in multiple doses.

10 Further, the LHRH embodiments relate to a method for improving the organoleptic qualities and tenderness of the meat from male domestic animals while maintaining the advantageous growth performance of intact males. The androgenic steroid hormones of intact males are responsible
15 for fast growth but their presence is accompanied by non-androgenic steroids (e.g., 5 α androstene-3-one) and skatole (a product of the microbial metabolism of tryptophan) which impart unpleasant taste and aroma to the meat. This condition, known as boar taint in the case of swine, detracts
20 from the quality of the meat. However, by the active immunization of young males with compositions comprising LHRH peptides of the invention, on a schedule that effects immunocastration in the weeks just prior to slaughter, many of the growth advantages of non-castrated males may be retained
25 while providing meat with improved flavor and tenderness.

The efficacy of the peptide composition of the present invention comprising the target antigenic site, LHRH, can be tested by the procedure described in the Examples 1-3.

Other target antigenic sites which have also been
30 used in peptide immunogens of the present invention are described in Examples 4-9. Peptide immunogen compositions are useful for inducing immune responses in mammals against specific target antigens and provide for prevention or treatment of disease, or intervene to usefully modify normal
35 physiological conditions.

EXAMPLE 1IMMUNIZATION OF RATS WITH PEPTIDE
IMMUNOGENS CONTAINING LHRH

5 Peptides listed in Tables 2a and 2b were synthesized and tested as described below.

 A. Peptide synthesis. The peptides listed in Tables 2a and 2b were synthesized individually by the Merrifield solid-phase synthesis technique on Applied Biosystems automated peptide synthesizers (Models 430, 431 and 433A) using Fmoc chemistry. Preparation of peptide constructs comprising structured synthetic antigen libraries (SSALs), e.g., the artificial Th site designated SEQ ID NO:6-8, was accomplished by providing a mixture of the desired amino acids selected for a given position. After complete assembly of the desired peptide or combinatorial peptides, the resin was treated according to standard procedure using trifluoroacetic acid to cleave the peptide from the resin and deblock the protecting groups on the amino acid side chains.

20 The cleaved, extracted and washed peptides were purified by HPLC and characterized by mass spectrometry and reverse phase HPLC.

 Peptides were synthesized to have the LHRH target antigenic peptide (SEQ ID NO:77) in tandem with each of the designed Th epitopes as listed in Tables 2a and 2b. The Th epitopes were those shown in Tables 1a and 1b (SEQ ID NOS:6, 12-19, 105, 20-22 and 31-35). For purposes of comparison, prior art peptide immunogens comprising model Th sites (SEQ ID NOS:36 and 65), and prototype Th sites (SEQ ID NOS:37-40 and 66-70) and a peptide/carrier protein conjugate, KLH-LHRH (Table 2b) were also synthesized and tested. The Th/LHRH and Inv/Th/LHRH peptide constructs were synthesized with gly-gly as a spacer between the target antigenic site and the Th epitope, and with or without gly-gly as a spacer between the Th epitope and the Inv immunostimulatory sequence. In

The results for peptide immunogens SEQ ID NOS:80-82 are not yet available.

10 Animals: Sprague-Dawley rats, male
 Group Size: 5-10 rats/group
 Immunogen: individual peptide immunogen
 Dose: amount in µg as specified, in 0.5mL
 Adjuvants: (1) Freund's Incomplete Adjuvant (IFA);
 or
 (2) Alum (Aluminum hydroxide);
15 One adjuvant per immunogen per group

20 Route: intramuscular

C. Method for determination of immunogenicity.

25 Antibody activities were determined by ELISA (enzyme-linked immunosorbent assays) using 96-well flat bottom microtiter plates which were coated with the LHRH peptide (SEQ ID NO:77) as immunosorbent. Aliquots (100 μ L) of the peptide immunogen solution at a concentration of 5 μ g/mL were incubated for 1
30 hour at 37°C. The plates were blocked by another incubation at 37°C for 1 hour with a 3% gelatin/PBS solution. The blocked plates were then dried and used for the assay. Aliquots (100 μ L) of the test immune sera, starting with a 1:100 dilution in a sample dilution buffer and ten-fold serial
35 dilutions thereafter, were added to the peptide coated plates.

The plates were incubated for 1 hour at 37°C.

The plates were washed six times with 0.05% TWEEN® in PBS. 100µL of horseradish peroxidase labeled goat-anti-rat IgG antibody was added at appropriate dilutions in conjugate dilution buffer (Phosphate buffer containing 0.5M NaCl, and normal goat serum). The plates were incubated for 1 hour at 37°C before being washed as above. Aliquots (100µL) of o-phenylenediamine substrate solution were then added. The color was allowed to develop for 5-15 minutes before the enzymatic color reaction was stopped by the addition of 50µL 2N H₂SO₄. The A_{492nm} of the contents of each well was read in a plate reader. ELISA titers were calculated based on linear regression analysis of the absorbances, with cutoff A_{492nm} set at 0.5. This cutoff value was rigorous as the values for diluted normal control samples run with each assay were less than 0.15.

D. Determination of immunogen efficacy. Immunogens were evaluated for efficacy by RIA for serum testosterone values. Serum testosterone levels were measured using an RIA kit from Diagnostic Products (Los Angeles, CA) according to manufacturer's instructions. The lower detection limit for testosterone ranged from 0.01 to 0.03nmol/L. Each sample was analyzed in duplicate. Serum samples were scored as being at castration level when the testosterone level was below limits of detection and as "near castration" at <0.1nmol/L. Results were verified by comparison to testosterone levels in serum from mechanically castrated rats.

E. Results. Results from serum samples collected at weeks 10 or 12 are presented in Tables 2a and 2b. (The peptides of the Tables are ordered by derivation of their Th epitopes, as was done in Tables 1a and 1b.) ELISA data (not shown) demonstrated that immunization by all the listed immunogens resulted in antibody responses in all animals. The efficacy of the anti-peptide antibody responses, consequential to the cross-reactivity to natural LHRH, was established by

determining serum testosterone levels. Those results are summarized in the right columns of Tables 2a and 2b as numbers of animals having castration level serum testosterone per total animals in the group.

5 The results show that the peptides of the invention, whether with a strong adjuvant IFA and administered 3 times at high dose, or with a weak adjuvant Alum and administered twice at low dose were effective in producing immunocastration. The immunogenicity of the Th sites SEQ ID NOS:6, 9 and 15 were
10 improved by the addition of the Inv domain sequence. See comparisons between SEQ ID NOS:41, 44 and 45, 46 and SEQ ID NOS:53 and 60. Although, the addition of the Inv domain sequence did not always result in improvement in immunogenicity, e.g., compare SEQ ID NOS:51 and 52, SEQ ID
15 NOS:61 and 62, and, SEQ ID NOS:74 and 75. Two peptides of the invention (SEQ ID NOS:50 and 76) were tested only at low dose with the weak adjuvant and failed to cause immunocastration, but the results with other peptides, e.g., SEQ ID NO:73, indicate that they would have been effective at a higher dose
20 with a strong adjuvant. Many of the LHRH peptide immunogens of the present invention were significantly more effective at inducing immunocastration than the KLH/LHRH peptide carrier protein conjugate or the peptide immunogens having HBsAg Th (SEQ ID Nos:65 and 66-70). See Table 2b.

25 Also, the peptide immunogens of the present invention were more easily synthesized than the peptide/carrier conjugate protein or the peptide immunogens having the more complex prototype Th epitopes of the prior art (SEQ ID NOS:2-5 or 26-30). Yet, equivalent or improved
30 immunogenicity with fewer and lower doses were obtained with the peptide immunogens comprising the artificial Th epitopes of the present invention.

 A serological analysis of the antibody responses of rats that had received the LHRH peptides of the invention
35 demonstrated that the antibody responses to the peptides was specifically directed to the target antigenic site and not to

- 30 -

the novel artificial Th sites. This is a distinct advantage of these peptide immunogens over conventional peptide/carrier protein conjugates. Serum samples from rats that had been immunized with the peptide immunogens shown in Table 3, with 25µg doses on Alum at 0 and 3 weeks, were compared for reactivities to the LHRH target site and to the Th epitope by ELISAs using the LHRH peptide (SEQ ID NO:77) and the appropriate Th epitope (SEQ ID NO:15 and 18, 31, or 34) as solid-phase substrates in peptide-based ELISAs. Results for these ELISAs are presented in Table 3 which show that despite high titer responsiveness to the LHRH moiety of the Th/LHRH peptide conjugates, reactivities for the artificial Th sites were at background levels.

EXAMPLE 2LHRH PEPTIDE MIXTURE FOR INDUCTION OF BROADER
IMMUNOCASTRATION IN RATS

5 Establishing the relative efficacies of the various
artificial Th epitope/LHRH constructs as shown above in
Example 1 permitted selection of the most effective ones for
assembly into a peptide mixture of enhanced immunogenicity. A
mixture of Th/LHRH peptide immunogens is more efficacious than
10 any individual peptide within the mixture (US 5,795,551).
Moreover, a mixture of individual constructs carrying
promiscuous Th epitopes derived from MVF Th (SEQ ID NO:1) and
HBsAg Th (SEQ ID NOS:23-25) provide broader response in a
genetically diverse population than would a peptide
15 composition having Th epitopes derived from only one
promiscuous Th epitope. Therefore, a peptide composition
comprising a mixture of peptides of the invention derived from
MVF Th and HBsAg Th was assembled and the efficacy of the
mixture was tested and compared to compositions comprising the
20 individual peptides of the mixture.

Groups of 6 or 8 male rats were immunized with 25µg
doses (total dose) of the peptide compositions indicated in
Table 4. The peptides in the mixture were combined in
equimolar proportions. The peptides were formulated with 0.4%
25 Alum and administered intramuscularly on weeks 0 and 3. Serum
testosterone levels were followed for 22 weeks and the results
were scored as number of animals with castration level of
testosterone per total number of animals in the group. These
results are presented in Table 4. They demonstrated that the
30 low doses of peptide compositions, given with a relatively
ineffective adjuvant, achieved castration levels of
testosterone by week 5, and that this response was maintained
through week 22. Moreover, the peptide mixture performed
significantly better than one of the peptide compositions
35 comprising an individual peptide. It can be assumed that the

mixture would have shown improved immunogenicity over the other individual peptide composition had the numbers of experimental animals been larger and more representative of a true population.

5

EXAMPLE 3LHRH PEPTIDE MIXTURE AND FORMULATIONS FOR
THE IMMUNOCASTRATION OF SWINE

10 A group test animals have been shown to be more broadly responsive to a mixture of peptide immunogens with different Th epitopes than to a composition containing a single peptide immunogen. However, for the prevention of boar taint in swine, it is necessary that the immunopotent LHRH
15 peptide immunogens be sufficiently potent to elicit the desired response in most animals while being acceptable for use in food animals. It is important that there is no immediate effect adverse to the growth rate and that no residue of the peptide immunogen or the adjuvant is left in
20 the meat or cause lesions in the marketable parts of the carcass.

In order to evaluate the useful immunogenicity of a mixture of inventive LHRH peptides, the mixture was administered to swine in three formulations either in 0.4%
25 Alum, IFA, or ISA 206/DDA. ISA 206/DDA is an oil/water emulsion in which Dimethyldioctadecylammonium bromide (DDA) is dispersed into MONTANIDE® ISA 206 at 30mg/mL (MONTANIDE® ISA 206 is an oily metabolizable solution supplied by SEPPIC Inc. of Fairfield, NJ). The oil suspension is then emulsified at a
30 1:1 volume ratio into an aqueous peptide solution which has been adjusted for peptide concentration so as to provide the desired dose of peptide in 0.5mL of the final preparation.

The immunization protocol was as follows:

Animals: Yorkshire Hampshire Cross Swine, males,

- 33 -

3-4 weeks of age non-castrated
Group Size: 2-3 animals/group
Immunogen: Equimolar mixture of SEQ ID NOS:57-58, 71
and 75
5 Dose: 400µg of peptide(s) in 0.5mL
Adjuvants: (1) 0.4% Alum,
(2) IFA,
(3) ISA 206/DDA
Schedule: 0, 4, and 13 weeks or 0, 4 weeks
10 Route: Intramuscular

The efficacy of the peptide immunogen formulations was monitored by assaying the swine serum samples collected throughout the course of the study, the results are graphically presented in Figs 1-3. The assays included an RIA
15 for the determination of the presence of antibodies cross-reactive to native LHRH in solution as described below, and an RIA for testosterone as described in EXAMPLE 1. Further, the average testes cross sectional area was determined by palpitation with a caliper.

20 Antisera for the anti-LHRH RIA were diluted 1:100 in 1% bovine serum albumin (BSA), pH 7.4. An equal volume of diluted sera was added to 100µL of [¹²⁵I]-LHRH (New England Nuclear Company, Boston, MA) diluted in 1% BSA to contain approximately 15,000cpm for 5.25pg LHRH. The solution was
25 incubated overnight at room temperature and antibody-bound LHRH was precipitated with 400µL of 25% polyethylene glycol (MW 8,000) in 0.01M phosphate-buffered saline (PBS), pH 7.6, and 200µL of 5mg/mL bovine gamma globulin in PBS. Anti-LHRH antibody concentrations are expressed as nmol iodinated LHRH
30 bound per liter of serum (Ladd et al., 1988, *Am J Reprod Immunol*, 17:121-127).

Results are depicted graphically in Figs 1-3 for the three assays. The intervals at which the immunogens were administered are shown by arrows at the bottom of the graphs.
35 Determinations for the individual experimental animals in

each figure are represented by the solid circles, triangles, and squares. The swine responded immunologically to all three formulations, as shown by the presence of anti-LHRH antibody and the concomitant suppression of testosterone.

5 The alum preparation (Fig. 1) was least effective producing a lower level of antibody responses. One animal of this group did not achieve the castration level of testosterone until week 11 and both animals in this group did not manifest complete involution of the testes. The animals
10 of the alum group did not receive immunizations at week 13, and the effects of the treatment were reversed.

 The animals of the IFA group (Fig. 2) displayed higher levels of antibody responses, with two of the three reaching and holding a castration level of testosterone by
15 week 6. However, upon administration of a booster dose at week 13, the lowest responding swine of the three failed to respond and reverted to a normal level of testosterone and to non-involuted testes. The two responsive animals of this group achieved complete involution of the testes by week 23.

20 Both swine of the ISA 206/DDA group (Fig. 3) provided high and relatively uniform levels of antibody responses. Immunocastration levels of testosterone in this group were achieved by week 9 and stably maintained through week 12. Both animals were responsive to the boost at week 13
25 and maintained castration levels of testosterone. The testes of both animals were undetectable by week 23.

 From the results obtained the ISA 206/DDA formulation is, thus, most preferred for prevention of boar taint. High and uniform effects on the two animals are
30 achieved with the ISA 206/DDA formulation. Moreover, the formulation is more acceptable in swine in comparison to the IFA formulation which caused lesions, apparently because the IFA formulation is not readily metabolized.

EXAMPLE 4

FOR GROWTH PROMOTION IN FARM ANIMALS

Immunogens of the invention may be used singly or in combination to elicit antibodies to somatostatin.

Somatostatin is a major inhibitor of total somatic growth. It is a cyclic peptide hormone of fourteen amino acids (SEQ ID NO:80, Table 5) and its structure is conserved across species.

Somatostatin inhibits the release of many gastro-intestinal hormones as well as inhibits the release of growth hormone, insulin, and thyroid hormones, thereby affecting both the ability of the animal to absorb nutrients and its subsequent ability to direct these nutrients into tissue growth. The neutralization of somatostatin by immunization has been shown to stimulate growth in sheep, goats, chickens, and pigs (Spencer, *Dom Anim Endocr*, 1986; 3:55; Spencer et al., *Reprod Nutr Develop*, 1987; 27(2B):581; Laarveld et al., *Can J Anim Sci*, 1986, 66:77), and cattle (Lawrence et al., *J Anim Sci*, 1986, 63(Suppl): 215).

In addition to stimulating growth rate and leading to a 20% reduction in rearing time (Spencer, 1986; Spencer et al., 1987) active immunization against somatostatin also has a beneficial effect the efficiency of food conversion, i.e., in addition to the saving on feed by virtue of more rapid growth, the animals actually utilize their food more efficiently during the growing period, at least partly as a result of changes in gut motility (Fadlalla et al., *J Anim Sci*, 1985, 61:234). The treatment does not have any marked effect on carcass composition (Spencer et al., 1987) but there were indications that, when killed at equal weights, treated animals may be less fatty and leaner. Taken all of the experimental data together, effective active immunization to somatostatin (as evidenced by the presence of anti-somatostatin antibodies) is a powerful, safe, and effective tool to enhance growth (Spencer, 1986).

However, somatostatin is a short peptide and a self-antigen and is non-immunogenic by itself (see Table 5).

Nevertheless, several immunogenic forms of somatostatin have been designed and tested as reported in the literature. For example, somatostatin has been conjugated with protein carriers to enhance immunopotency. However, protein carriers are too expensive for economical use in farm animals. Further, effective immunization with somatostatin is highly dependent on how the carrier is conjugated to somatostatin. In most cases, glutaraldehyde is employed as the carrier for coupling with the lysine residues present on somatostatin and glutaraldehyde. The two lysines on somatostatin available for coupling reside within a 12-mer functional loop. The conjugation of these lysines may result in significant loss of the native somatostatin structure. As a result, cross-reactivity to natural somatostatin with the antibodies is reduced. Moreover, with protein carriers, the majority of immune responses are directed to the carrier rather than to somatostatin (the mass of the carrier molecule(s) is much greater than that of somatostatin). Immunization with a small peptide carrier conjugates has frequently led to carrier-induced immune suppression (Schutz et al., *J Immunol*, 1985, 135:2319). Accordingly, there is a need for a different way to enhance the immunogenicity that is more suitable for farm animal use. The vaccine should be inexpensive and capable of stimulating an early and strong immune response to somatostatin and avoid carrier-induced suppression.

The somatostatin/Th epitope peptide immunogens shown in Table 5 were synthesized and administered to rats. The effect of the immunization was determined by peptide ELISA as described in EXAMPLE 1. Cyclized Somatostatin was used in one of the peptide immunogen (SEQ ID NO:80) tested and in the assay as the solid-phase substrate in ELISA. To completely cyclize somatostatin, the cleaved peptide was dissolved in 15% DMSO in water for 48 hrs to facilitate intra-disulfide bond formation.

From the results shown in Table 5, it is clear that somatostatin alone is devoid of immunogenicity whereas the

peptide immunogens of the present invention elicited high titers of somatostatin-specific antibodies in the immunized hosts. The anti-somatostatin responses generated for SEQ ID NO:81-83, SEQ ID NO:84-86 and SEQ ID NO:87, with Th epitopes (SEQ ID NOS:6,7,8 and 31) show the effectiveness of the Th epitopes. However, a close comparison of the antibodies titers for immunogenicity show that it is preferable to place the Th epitope on the C-terminus. The results for SEQ ID NOS:84-86, with the Th epitope as SEQ ID NOS:6,7,8 show an earlier higher level of antibodies was elicited. The results of Table 5 illustrate the strong antibody response to immunization with artificial Th/somatostatin compositions, thereby establishing the utility of these peptides of this invention for growth promotion in farm animals.

EXAMPLE 5

PEPTIDE COMPOSITION FOR PREVENTION OF HIV INFECTION

Peptide immunogens comprising idealized artificial Th sites of the present invention and a target antigenic site cross-reactive to a host cell receptor/co-receptor complex for HIV may be used to elicit antibodies to that host cell complex in the immunized host. Said complex which is located on the surface of host lymphocytes expressing CD4 comprise CD4 associated with a chemokine receptor domain. This complex is the primary receptor for entry of HIV into T cells. Antibodies directed to this CD4 complex block the interactions between HIV and its receptor, and the interactions between CD4-Class II and CD4-expressing T cells and other activated T cells. Thus, antibodies directed to this complex have broad neutralizing activities against primary isolates of HIV-1, HIV-2, and SIV and intervene in the immunosuppression of CD4+ cell-mediated immune responses (WO 97/46697).

The peptide immunogens relevant to the CD4 complex antigenic site may be formulated singly or in combination for the generation, by active immunization in mammals including

humans, high titers of serum antibodies to the CD4 complex. These antibodies are useful for the prevention and treatment of immunodeficiency virus infection as well as for treatment of undesirable immune responses such as transplant rejection, rheumatoid arthritis, systemic lupus erythematosus, and psoriasis.

An artificial Th epitope/target antigenic site peptide immunogen was designed with a sequence modified from the CDR2-like domain of CD4 as the target antigenic site. The modified site comprises a peptide sequence taken from the CDR2-like domain of human CD4 (amino acids 39-66 according to the numbering system of Maddon et al., Cell, 1985; 42:93; and, Littman et al., Cell, 1988; 55:541) modified as follows: (1) the insertion of cysteine residue to the N-terminus side of position 39 of the naturally occurring CD4 sequences, (2) the insertion of a cysteine residue at the C-terminus side of position 66 thereof, and (3) the formation of a disulfide bond between the inserted cysteines to produce a cyclic structure.

The optimized and modified (i.e., cyclized) target site for CD4-CDR2 of the following sequence is provided:

1. Cys-Asn-Gln-Gly-Ser-Phe-Leu-Thr-Lys-Gly-Pro-Ser-Lys-Leu-Asn-Asp-Arg-Ala-Asp-Ser-Arg-Arg-Ser-Leu-Trp-Asp-Gln-Gly-Asn-Cys
2. (SEQ ID NO:88)

To complete the cyclization, the modified peptide was dissolved in 15% DMSO in water for 48 hrs to facilitate intra-disulfide bond formation between the cysteines. SEQ ID NO:84 was incorporated into the peptide immunogen:

(SEQ ID NO:6,7,8)-GG-(SEQ ID NO:88)

(SEQ ID NO:89-91)

Immunogenicity in guinea pigs of SEQ ID NO:85 formulated in ISA 206/DDA, 100 µg/dose, given at weeks 0, 3, 6, was evaluated. Immunogenicity was determined by peptide ELISA as described in EXAMPLE 1. The cyclized target antigenic site peptide used as the solid-phase substrate in the ELISA. The labeled conjugate was specific for guinea pig IgG. Six of

six guinea pigs were successfully seroconverted by the ELISA reactivity obtained. It is significant that SEQ ID NO:85 was found to be highly immunogenic and is functional in a large animal.

- 5 An immunogenic composition comprising SEQ ID NO:85 was formulated in IFA, 300µg/dose, and administered to a swine by intramuscular injection on weeks 0, 3, and 6. The swine seroconverted and the serum from week 8 was tested for neutralization activity against a primary isolate of HIV-1.
- 10 Neutralization activity was assayed on HIV-1 VL135, a primary isolate of subtype B, by MT-2 Microplaque Neutralization Assay (Hanson et al., J Clin Microbiol, 1990; 28:2030; WO 97/46697).
- 15 The swine serum sample provided 50% virus neutralization at a dilution of 1:249, and 90% neutralization at a dilution of 1:97. Therefore, immunization of a large animal host with a peptide immunogen composition of the present invention produced antibodies which bind to the host cell receptor comprising CD4 and neutralize HIV.

EXAMPLE 6PEPTIDE COMPOSITION FOR TREATMENT OF ALLERGY

Peptide immunogens comprising the idealized

5 artificial Th sites of the invention and a target antigenic site that cross-reacts with an effector site on the third constant domain (CH3) of the epsilon (ϵ) heavy chain of IgE are provided. The immunogens may be used to elicit auto-antibodies to the IgE effector site in the immunized host.

10 That IgE-CH3 effector site is modified from a segment of the CH3 domain of the epsilon (ϵ) heavy chain of human IgE (amino acids 413-435 (Dorrington and Bennich, 1978; 41:3). It is modified from that of the naturally occurring IgE sequence as follows: (1) insertion of a cysteine residue to the N-

15 terminus side at position 413, (2) substitution for the native cysteine at position 418 of the native IgE sequence by serine, (3) insertion of cysteine at C-terminus side at position 435, and (4) formation of a disulfide bond between the cysteines at the N- and C- termini to produce a cyclic structure. By this

20 process the target antigen site for human IgE is optimized.

3. **Cys**-Gly-Glu-Thr-Tyr-Gln-Ser-Arg-Val-Thr-His-Pro-His-

4. Leu-Pro-Arg-Ala-Leu-Met-Arg-Ser-Thr-Thr-Lys-**Cys**

(SEQ ID NO.:92)

Amino acid substitutions from the natural sequence

25 are shown in boldface. The results show that the polyclonal antibodies elicited have specificity for the CH3 effector site of IgE in the immunized host. They prevent the sensitization of mast cells and basophils by IgE, thereby preventing the triggering and activation of mast cells/basophils and leading

30 to the down-regulation of IgE synthesis. Moreover, antibodies elicited by peptides shown in Table 6 comprising this target antigenic site are cross-reactive with human IgE, and these antibodies are safe and non-anaphylactogenic. Furthermore, the antibodies do not crosslink to IgE bound to the high

affinity cell receptor to induce degranulation.

The peptide conjugates relevant to the IgE-CH3 antigenic site may be formulated singly or in combination and used to immunize mammals including humans to generate high
5 titers of serum antibodies, which are useful for the prevention and treatment of allergic symptoms.

The peptide immunogens incorporating the modified IgE-CH3 target site (SEQ ID NO:92) are shown in Table 6 as SEQ ID NOS:87-90. These peptide immunogens were used to immunize
10 groups of 3 guinea pigs using 100µg/dose, formulated in CFA at week 0, IFA at weeks 3 and 6, and administered intramuscularly. For comparison, a group of two animals were immunized with a peptide/KLH carrier protein conjugate at 200µg/dose, similarly administered. ELISA results of serum
15 samples collected at week 8 are shown. In the ELISA a human IgE myeloma protein (American Biosystem, Inc. cat. no. A113) was used as the solid-phase immunoabsorbant. The procedure used was identical to the peptide-based ELISAs described previously, except IgE myeloma was used as the solid phase
20 immunoabsorbant. Thus, the results demonstrate cross-reactivity with human IgE. The ELISA results also demonstrate that all of the constructs were immunogenic with cross-reactivity for human IgE, with the peptide immunogens of the present invention providing superior immunogenicity. The
25 inclusion of the Inv domain sequence (SEQ ID NO:78) augmented the immunogenicity, as shown by the enhanced immunogenicity of SEQ ID NO:99 over SEQ ID NO:98.

To test the anti-human IgE antibodies in assays for biological activity and safety, guinea pig hyperimmune sera
30 were produced against SEQ ID NO:98 and 99. The procedure is as described above, except that the animals also received a booster dose of the peptide immunogen in IFA on week 10. Guinea pig IgG antibodies were purified and the ability of the purified antibodies to inhibit the sensitization of human
35 basophils by allergen-specific IgE determined as follows.

Guinea pig IgG antibodies were purified by Protein A affinity chromatography (ImmunoPure[®] Immobilized Recomb[®] Protein A, Pierce) from serum collected at weeks 8 and 12. The serum from the animals immunized with SEQ ID NOS 98, 99 were pooled. The eluted antibodies were prepared at a standard concentration of 8mg/mL in 25mM PIPES buffer, 0.15M NaCl, pH 7.2. A control antibody preparation was prepared from the pooled serum of guinea pigs immunized with an irrelevant peptide immunogen. These antibodies were used in assays that measure the reduction in IgE-mediated sensitization of human basophils. Human basophils were prepared from the venous blood of volunteers using centrifugation through Percoll density gradients (MacGlashan. J Allergy Clin Immunol, 1993; 91:605-615). The banded leukocytes were collected, washed, and resuspended in 0.1mL of PAGCM buffer as describe (MacGlashan, 1993) except that the PAGCM buffer used to suspend the cells was made up with water containing 44% D₂O. The IgE used for the assay was allergen-specific, either human BPO-specific IgE or chimeric human IgE having grafted variable domains with specificity for HIV glycoprotein gp120. The allergen-specific IgE at 0.25µg/mL was preincubated for 15 minutes at 37°C with an equal volume of the purified antibody at 8mg/mL. The total volume was 0.1mL. The antibody mixture was added to the and incubated for 20 minutes to allow for sensitization of the basophils by uncomplexed IgE. The sensitized were then stimulated by addition of the allergen, either BPO₂₁-HSA or a gp120 polypeptide as described (MacGlashan, 1993). After an appropriate incubation period (usually 45 minutes), the basophils were separated from the supernatant and the supernatant assayed for histamine content by an automated fluorimetric technique (Siraganian, Anal Biochem, 1974; 57: 383-394). All reactions were performed in duplicate. The percent histamine release was calculated from the ratio of sample to total histamine minus from both the amount of spontaneous histamine release. The histamine

release by experimental antibody to histamine release by the control antibody of irrelevant specificity was compared and the ratio obtained. (Histamine release assays on human basophils were kindly performed under coded conditions by Dr. Donald W. MacGlashan, The Johns Hopkins University School of Medicine, Johns Hopkins Asthma and Allergy Center, Baltimore).

Specific inhibition of histamine release by the site-specific anti-IgE was 61% and 71% for the antibodies purified from bleeds taken on weeks 8 and 12, respectively.

10 These actively induced polyclonal antibodies were then further tested for safety. They were tested for ability or inability to cross-link receptor-bound IgE and induce spontaneous histamine release in the absence of allergen. This establishes whether or not they are non-anaphylactogenic
15 anti-IgE antibodies. A preparation of guinea pig anti-SEQ ID NO:98 was tested by direct challenge of IgE-sensitized basophils, in the absence of allergen, to evaluate its ability to crosslink receptor-bound IgE and induce degranulation. Histamine release by anti-SEQ ID NO:98 was equivalent to the
20 level of spontaneous histamine release by the donor cells. Based on this result, it was concluded that antibody of specificity for the target antigen site of SEQ ID NO:98, i.e., SEQ ID NO:92, is non-anaphylactogenic.

 The 12 week anti-SEQ ID NO:98 preparation was also
25 evaluated for IgE-specificity, to determine the potential of these antibodies for isotype specific immunosuppression. The cross-reactivity of anti-SEQ ID NO:98 guinea pig antibodies to human IgE and IgG were compared by ELISA. The procedure is described for human IgE. For human IgG ELISA, human IgG was
30 used as the solid-phase immunosorbent.

 The IgE ELISA plates were coated with the human IgE myeloma at 5µg/mL. For the IgG ELISA, the plates were coated with human purified IgG (Sigma reagent grade human IgG), also at 5µg/mL. The purified guinea pig anti-SEQ ID NO:98 was
35 tested for reactivity in both ELISAs at concentrations of 0.5

and 0.1µg/mL. Results were compared to antibodies purified from control guinea pig serum and to a "no antibody" control.

The A₄₉₀ values obtained for anti-SEQ ID NO:98 antibody on IgE were 1.126 at 0.5µg/mL and 0.344 at 0.1µg/mL. The A₄₉₀ values
5 obtained for anti-SEQ ID NO:98 antibody on IgG were equal to control antibody and background values. This shows that there was no cross reactivity of the guinea pig anti-SEQ ID NO:98 to human IgG.

The peptide immunogen composition of the present
10 invention did not elicit antibodies that recognize IgG antibodies, and therefore are isotype specific for IgE. Thus, it can be concluded that active immunization with Th/IgE target antigenic site immunogens of the invention elicit safe non-anaphylactogenic anti-IgE antibodies. The antibodies were
15 effective in inhibiting IgE-mediated sensitization, and displayed an immunosuppressive potential specific to antibodies of isotype IgE.

EXAMPLE 7PEPTIDE COMPOSITION FOR PREVENTION
OF FOOT-AND-MOUTH DISEASE

- 5 Peptide immunogens comprising idealized artificial
Th sites of the invention and a target antigenic site cross-
reactive to the "G-H Loop" on the VP1 capsid protein of Foot-
and Mouth Disease Virus (FMDV), may be used to elicit
neutralizing antibodies to FMDV.
- 10 Foot-and-mouth disease (FMD) is the most
economically important disease of domestic livestock. Cloven-
hoofed species including cattle, pigs, sheep and goats are
susceptible. Seven distinct serotypes have been described: A,
O, C, Asia, and the South African types SAT-1, 2, and 3, each
15 of which can be subdivided into multiple subtypes. Viruses of
serotypes A, O, and Asia-1 are most common. Serotype A
viruses are the most variable, having more than 30 subtypes.
There is no cross-protection between serotypes so that animals
recovered from infection with or vaccinated against a virus of
20 one serotype are still susceptible to infection with viruses
from the remaining six serotypes. Moreover, the degree of
antigenic variation within a serotype is such that a vaccine
made against one subtype may not be protective for another
subtype within that same serotype (Brown, Vaccine, 1992;
25 10:1022-1026).
- Serotype-specific peptides corresponding to the 141-
160 region (the G-H Loop) of the VP1 capsid proteins of
isolates belonging to all seven FMDV serotypes have been shown
to elicit protective levels of type-specific neutralizing
30 antibodies in guinea pigs (Francis et al., *Immunology*, 1990;
69:171-176). Clearly this region contains a dominant
immunogenic site which also carries the serotype specificity
of the virus. The initial observations of immunogenicity for
these 141-160 VP1 peptides was accomplished using synthetic

peptides conjugated to carrier protein KLH (keyhole limpet hemocyanin), a procedure which negates the advantage of manufacturing a well-defined synthetic immunogen. However, the development of VP1 synthetic immunogens was advanced by

5 DiMarchi and Brook (U.S. Patent No. 4,732,971) who showed that two VP1 sequences from a subtype O isolate, joined in a 200-213 Pro-Pro-Ser-141-158-Pro-Cys-Gly (SEQ ID NO:100) chimeric construct, elicited antibody levels which protected cattle

10 against challenge. Nevertheless, the efficacy of this effect was limited by the low immunogenicity of the peptide immunogen and by its narrow serotype specificity. Practical application demands that a vaccine formulation provide protection from both homotypic and heterotypic exposure, with small amounts of peptide immunogen.

15 The G-H Loop immunodominant site optimized for immunogenicity and capacity to induce broadly neutralizing antibodies is incorporated as a target antigenic site into the peptide immunogens of the present invention. The site is homologous to amino acid positions 134-169 on the FMDV VP1

20 protein of strain A12 (Robertson et al, *J Virol*, 1985; 54:651-660) and extend beyond either end of the G-H Loop. The target site was further modified by: (1) substitution of Asp at position 134 and Gln at position 157 with cysteines, (2) formation of a disulfide bond between the substituent

25 cysteines to produce a cyclic structure, and (3) construction of an SSAL thereof using the corresponding VP1 sequences from a selection of subtypes. The optimized target antigen sites for the FMDV VP1 are exemplified by the peptides listed in Table 7. The two peptide immunogens, SEQ ID NO:101 and SEQ ID

30 NO:102, are SSAL target antigenic sites compiled from strains of FMDV serotypes O and Asia, respectively. The target antigenic sites of both serotype O and Asia were coupled to the Th epitopes of the present invention, SEQ ID NO: 31. The Inv domain sequence (SEQ ID NO:78) was also incorporated into

35 SEQ ID NO:102.

These two SSAL peptide immunogens were synthesized and used to hyperimmunize groups of three Duncan Hartley guinea pigs (female, 9 weeks old, 450 gm, virus free). Each animal was immunized with 100µg per dose of the indicated synthetic construct emulsified in CFA at week 0 or IFA at weeks 3 and 6. The animals were bled on weeks 0, 5 and 10 for testing.

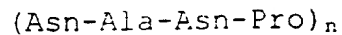
Serum samples were obtained from five and 10 week bleeds and pooled from each group. The pooled sera were evaluated for reactivity to the VP1 neutralizing epitope by peptide based ELISA using a peptide having the sequences for the neutralizing epitope (VP1 134-169) as the solid phase immunoabsorbant. Their ability to neutralize FMDV strains A12_{FP}, A_{FL}, A23, O-1_{JH}, O-1_{P2}, and Asia-1 were also determined. The virus neutralizing activity of a 1:100 dilution of a serum sample was determined by observing neutralization on a series of increasing input viral loads, using aliquots (10,000 MPD₅₀) of the virus strains named above. The results are shown in Table 7. The testing method performed (Morgan and Moore, Am J Vet Res, 1990; 51:40-45), demonstrated that immunization with the peptide antigens of the present invention provided sera which reduced by 2.5 Log₁₀ FMDV microplaques at 1:100 dilution. The results also are highly predictive of protective immunity against FMDV infection.

As shown by Table 7, high anti-peptide titers against the target antigenic sites (>5 Log₁₀) were elicited by week 5. Broad and effective neutralization of all seven tested strains belonging to three different FMDV serotypes (i.e., A, O, Asia) were observed, despite the wide variations between these strains and serotypes.

This further demonstrates the efficacy of the artificial Th epitopes of the present invention to stimulate effective antibody responses against an epitope from a foreign pathogen.

EXAMPLE 8PEPTIDE COMPOSITION OF A SPOROZOITE MALARIA VACCINE

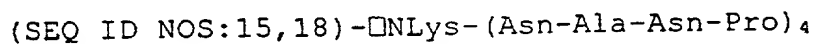
Peptide immunogens comprising the idealized artificial Th sites and a circumsporozoite (CS) target antigen from *Plasmodium falciparum*, a human malaria parasite, are provided. The CS protein is the major surface antigen of the sporozoite stage of the parasite. Immunologic studies and sequence data on a large number of CS genes of human, simian, and rodent plasmodia have been documented showing that all CS proteins contain a central region, consisting of a series of tandem repeats, encompassing multiple copies of an immunodominant B cell epitope. In *P. falciparum* the repeating epitope is represented as



(SEQ ID NO:103)

Antibodies directed against the repeats of the CS protein of the human malaria parasites, *P. falciparum* and *P. vivax*, inhibit the invasion of hepatocytes by sporozoites and abolish their infectivity. Therefore, repeats of the CS epitope have been the target antigen of subunit vaccines used in various human malaria vaccine trials (Nussenzweig et al., *Adv. Immunol.*, 1989, 45:283; Hoffman et al., *Science*, 1991, 252:520). However, one of the shortcomings of the various synthetic malaria vaccines currently in clinical trials is their low immunogenicity. Thus, a potential malaria vaccine remains to be developed. (Calvo-Calle et al., *J Immunol*, 1994, 150:1403)

To overcome the immunogenicity problem associated with the repeats of the CS protein of *P. falciparum*, the artificial Th epitopes shown in Table 1 are incorporated into the CS peptide immunogens. For example, peptide construct



(SEQ ID NOS:104,105)

is synthesized and used as the key immunogen in a malaria vaccine to elicit potent protective antibodies in

small animals, primates (e.g., baboons) and humans against *P. falciparum* sporozoites.

EXAMPLE 9

5 PEPTIDE COMPOSITION FOR PREVENTION AND TREATMENT OF
 ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASE

 Cholesteryl ester transport protein (CETP) mediates
the transfer of cholesteryl esters from HDL to TG-rich
lipoproteins such as VLDL and LDL, and also the reciprocal
10 exchange of TG from VLDL and LDL (Tall, *J Internal Med*, 1995,
237:5-12; Tall, *J Lipid Res*, 1993, 34:1255; Hesler et al., *J*
Biol Chem, 1987, 262:2275; Quig et al., *Ann Rev Nutr*, 1990,
10:169). CETP may play a role in modulating the levels of
cholesteryl esters and triglyceride associated with various
15 classes of lipoproteins. A high CETP cholesteryl ester
transfer activity has been correlated with increased levels of
LDL-associated cholesterol and VLDL-associated cholesterol,
which in turn are correlated with increased risk of
cardiovascular disease (see, e.g., Tato et al., *Arterioscler*
20 *Thromb Vascular Biol*, 1995, 15:112).

 CETP isolated from human plasma is a hydrophobic
glycoprotein having 476 amino acids. A cDNA encoding human
CETP has been cloned and sequenced (Drayna et al., *Nature*,
1987, 327:632). A monoclonal antibody TP2 (formerly
25 designated 5C7) has been produced which completely inhibits
the cholesteryl ester and triglyceride transfer activity of
CETP and, to a lesser extent, the phospholipid transfer
activity (Hesler et al., *J Biol Chem*, 1988, 263:5020). The
epitope of TP2 was localized to the carboxyl terminal 26 amino
30 acids, i.e., the amino acids from Arg451 to Ser476 of human
CETP (see, Hesler et al., 1988):

 Arg Asp Gly Phe Leu Leu Leu Gln Met Asp
 Phe Gly Phe Pro Glu His Leu Leu Val Asp
 Phe Leu Gln Ser Leu Ser

(SEQ ID NO:106)

TP2 was reported to inhibit both human and rabbit CETP activity in vitro and rabbit CETP in vivo (Yen et al., *J Clin Invest*, 1989, 83:2018). Further analysis of the region
5 of CETP bound by TP2 revealed that amino acids between Phe463 and Leu475

Phe Gly Phe Pro Glu His Leu Leu Val Asp Phe Leu Gln Ser Leu
Ser

(SEQ ID NO:107)

10 are necessary for TP2 binding and for neutralizing CETP neutral lipid binding and transfer activity (see, Wang et al., *J Biol Chem*, 1992, 270:612).

A number of in vivo studies utilizing animal models or humans have indicated that CETP activity can affect the
15 level of circulating cholesterol-containing HDL. Increased CETP cholesteryl ester transfer activity can produce a decrease in HDL-C levels relative to LDLC and/or VLDLC levels and low HDL which in turn is correlated with an increased susceptibility to arteriosclerosis. Therefore, the discovery
20 of compounds and methods to control CETP activity will be advantageous in preventing or treating cardiovascular disease.

Rittershaus et al., WO 96/34888, proposed the use of a peptide composition comprising a "universal" or "broad range" immunogenic helper T cell epitope linked, preferably
25 covalently, to a B cell epitope portion such as the one found in the carboxyl terminal portion of human CETP involved in a neutral lipid binding or transfer activity of CETP. In order to enhance the immunogenicity of the functional site on human CETP, i.e., the carboxyl terminal amino acids of human CETP
30 (SEQ ID NOS:96 and 97) and peptide analogues thereof linked to the antigenic peptides comprising artificial Th epitopes from Table 1 and CETP target antigen peptides were synthesized. These peptide antigens, shown in Table 8, are used to elicit antibodies to CETP in small animals, primates and humans.
35 Anti-CETP antibodies control CETP activity. Thereby, the CETP antigenic peptides are expected to cause reduced accumulation

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in plasma of LDL-associated cholesterol and provide protection from and treatment of arteriosclerosis and coronary heart disease.

EXAMPLE 10

5 PEPTIDE COMPOSITION AS HIV VACCINE COMPONENT FOR THE ELICITATION OF NEUTRALIZING ANTIBODIES AGAINST HIV

Progress in the development of an effective vaccine for HIV-1 has been gauged in large part by measuring the
10 ability to induce measurable virus-specific CD8⁺ cytotoxic T lymphocytes (CTLs) and neutralizing antibodies, the leading indicators of a protective immune response. A strong correlation was observed between protection against infection and levels of neutralizing Abs in nonhuman primates infected
15 with HIV-1 or simian HIV (SHIV). However, little progress has been made in generating neutralizing antibodies over the past decades. Part of the problem is the weak neutralizing antibody response generated by candidate HIV-1 vaccines. A recent article entitled "Toward an HIV Type 1 vaccine that
20 generates potent, broadly cross-reactive neutralizing antibodies" by Montefiori et al (*AIDS Res and Hum Retrovir*, 1999, 15:689-698) provides a state-of-the-art review of the difficulties encountered in HIV vaccine development.

In an effort to enhance the neutralizing antibody
25 response against HIV, we embarked on synthetic antigen designs aimed at eliciting potent neutralizing antibodies. The synthetic antigens employ specially designed artificial Th epitopes as the immune stimulatory element of the construct covalently linked to HIV neutralizing B cell epitopes (e.g.
30 Korber et al, *HIV Mol Immunol Database* 1997, Part III Antibody Binding Sites) or neutralizing mimetopes (e.g. Scala et al, *J Immunol* 1999, 162: 6155-6161) previously recognized by either monoclonal or polyclonal antibodies.

Amongst the HIV neutralizing B cell epitopes, an
35 artificial V3 consensus sequence was designed (SEQ ID NO: 125)

comprising an optimal frame of the V3 principal neutralizing determinant of HIV gp120 (Wang, C.Y., US 5,763,160). These include the most frequent amino acid for each of the amino acid positions within that frame based on analyses of amino acid sequences from over 1,000 HIV isolates from subtypes A to G, along with sequences derived from linear or conformational epitopes of HIV gp120/ gp41 regions shown here in Tables 9 and 10 in SEQ ID NOS:125, 126-129, 148-153. The neutralizing mimetopes of Scala et al (SEQ ID NOS:130-135) are also synthesized as peptides of the invention, SEQ ID NOS:136-147, as shown in Table 9.

The immunogenic peptide constructs of the invention shown in Table 10 are wholly synthetic and were synthesized by the solid-phase method outlined in Example 1. These immunogenic peptide constructs further illustrate the utility of the artificial Th of the present invention in HIV vaccine development.

Each peptide in Table 10 can be represented by the formula $(A)_n-(Th)_m-(B)_o-(HIV\ B\ neutralizing\ epitope)-X$ or $(HIV\ neutralizing\ epitope)-(B)_o-(Th)_m-(A)_n-X$. The HIV neutralizing epitopes include SEQ ID NOS: 125 and 130-135. The immunogenic peptides comprise one or more Th sites derived from artificial Th (as shown in Table 1). Each peptide of this example have Gly-Gly or $(\epsilon-N)Lys$ spacers between immunogenic elements, but peptides of the invention may have other spacers or no spacers.

Peptides of these examples may comprise an optional Inv immunostimulatory site (SEQ ID NO: 27). It is understood however that the invention is not limited to the use of Inv as an additional immunostimulatory element.

Representative peptide constructs of the invention as listed in Table 10 (SEQ ID NOS: 148-153) were synthesized, cleaved, cyclized and purified as described in Example 1. Each peptide construct was formulated for immunization into small animals such as guinea pigs, or into larger animals such

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as pigs or baboons for evaluation of its immunogenicity. Each of the peptides was suspended in a volume of 0.5 mL containing representative emulsifiers or adjuvants such as ISA51, ISA720, DDA or monophosphoryl lipid A (MPL). The dose was 100 µg of peptide for guinea pigs or 300 µg of peptide for swine or baboons and the animals were immunized intramuscularly.

Animals received injections on weeks 0, 3 and 6 as specified in Table 10. Test bleeds at 5, 8, 10 and 11 weeks post initial immunization were evaluated for reactivities with target epitopes by B cell epitope peptide ELISA as described in Example 1, and further tested for their ability to neutralize HIV-1 as described in details in Example 5.

All peptides tested elicited strong site-directed cross reactivities to the corresponding target peptide, as shown by Log₁₀ titers on the anti-B epitope peptide ELISAs of greater than 4. Neutralization of HIV-1 was also observed for immune sera obtained from guinea pigs, and baboons. This functional reactivity by the baboon sera is noteworthy insomuch as the neutralization of human HIV by the baboon sera is nearly a human system. The results are strong indicators of the efficacy of a peptide construct of the invention as an agent for the prevention and/or immunotherapy of HIV infection by active immunization.

Table 1

Model, Prototype, and Artificial Idealized Th Epitopes

a. MVF Th and Th epitopes derived therefrom

Th Identifier		Amino Acid Sequence
MVF Th	SEQ ID NO:1	LSEIKGVIVHRLEGV
SSAL1 Th1	SEQ ID NO:2	DLSDLK <u>GLLL</u> HKLDGL
	SEQ ID NO:3	EI EIR <u>III</u> RIE I
	SEQ ID NO:4	V V <u>VVV</u> V V
	SEQ ID NO:5	F F <u>FFF</u> F F
SEQ ID NO:6		ISEIKGVIVHKIEGI
SEQ ID NO:7		MT RT TRM TM
SEQ ID NO:8		L L V
SEQ ID NO:6		ISEIKGVIVHKIEGI
SEQ ID NO:9		T RT TR T
SEQ ID NO:10		MSEIKGVIVHKLEGM
SEQ ID NO:11		LT MRT TRM TV
SEQ ID NO:6		ISEIKGVIVHKIEGI
SEQ ID NO:12		ITEIRTVIVTRJETI
SEQ ID NO:13		MSEMKGIVIVHKMEGM
SEQ ID NO:14		LTEIRTVIVTRLETV
SEQ ID NO:15		ISISEIKGVIVHKIEGILF
SEQ ID NO:16		MT RT TRM TM
SEQ ID NO:17		L L V
SEQ ID NO:15		ISISEIKGVIVHKIEGILF
SEQ ID NO:18		T RT TR T
SEQ ID NO:19		ISLSEIKGVIVHKLEGMLF
SEQ ID NO:105		MT MRT TRM TV
SEQ ID NO:123		ISLTEIRTVIVTRLETVLF
SEQ ID NO:124		I I I
SEQ ID NO:15		ISISEIKGVIVHKIEGILF
SEQ ID NO:20		ISITEIRTVIVTRJETILF
SEQ ID NO:21		ISMSEMKGIVIVHKMEGMLF
SEQ ID NO:22		ISLTEIRTVIVTRLETVLF

b. HBsAg Th, Prototype and Derivatives

Th Identifier		Amino Acid Sequence
HbsAg.Th	SEQ ID NO:23	FFLLTRILTIQSLD
	SEQ ID NO:24	KKKFFLLTRILTIQSLD
	SEQ ID NO:25	FFLLTRILTIQSL
SSAL2 Th2	SEQ ID NO:26	KKKL <u>FL</u> LTKLLTLQSLD
	SEQ ID NO:27	RRRIK <u>II</u> RII I L IR
	SEQ ID NO:28	VR <u>VV</u> <u>VV</u> V I V
	SEQ ID NO:29	F <u>FF</u> <u>FF</u> F V F
	SEQ ID NO:30	F
SEQ ID NO:31		KKKIITITRIITIITTID
SEQ ID NO:32		KKKIITITRIITIITTI
SEQ ID NO:33		KKKMMTMRMITMITTID
SEQ ID NO:34		FITMDTKFLLASTHIL
SEQ ID NO:35		KKKFITMDTKFLLASTHIL

Table 2
Immunogenicity of LHRH Peptides

a. MVF Th Derivatives

SEQ ID NO:	Description of Antigenic Peptide	Formulations			
		no. castrated		no. castrated	
36	(SEQ ID NO:1)-GG-(LHRH) ^a	400 µg/dose IFA (0,3,6wpi)	8/10	400 µg/dose Alum (0,3,6wpi)	5/5
37	(SEQ ID NO:2)-GG-(LHRH) ^a	400 µg/dose IFA (0,3,6wpi)	9/10	400 µg/dose Alum (0,3,6wpi)	2/5
38	(SEQ ID NO:3)-GG-(LHRH) ^a				
39	(SEQ ID NO:4)-GG-(LHRH) ^a				
40	(SEQ ID NO:5)-GG-(LHRH) ^a				
41	(SEQ ID NO:6)-GG-(LHRH) ^a	400 µg/dose) IFA (0,3,6wpi)	6/6	25 µg/dose Alum (0,3,6wpi)	3/8
42	(SEQ ID NO:7)-GG-(LHRH) ^a				
43	(SEQ ID NO:8)-GG-(LHRH) ^a				
41	(SEQ ID NO:6)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3,6wpi)	1/6
44	(SEQ ID NO:9)-GG-(LHRH) ^a				
45	(Inv) ^a -GG-(SEQ ID NO:6)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	5/5
46	(Inv) ^b -GG-(SEQ ID NO:9)-GG-(LHRH) ^a				
47	(SEQ ID NO:10)-GG-(LHRH) ^a	100 µg/dose IFA (0,3,6wpi)	4/6	25 µg/dose Alum (0,3 wpi)	1/8
48	(SEQ ID NO:11)-GG-(LHRH) ^a				
45	(Inv) ^a -GG-(SEQ ID NO:6)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	2/6
49	(SEQ ID NO:12)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	5/6
50	(SEQ ID NO:13)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	0/6
51	(SEQ ID NO:14)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	5/6
52	(Inv) ^a -GG-(SEQ ID NO:14)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	3/6
53	(SEQ ID NO:15)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	4/8
54	(SEQ ID NO:16)-GG-(LHRH) ^a				
55	(SEQ ID NO:17)-GG-(LHRH) ^a				
53	(SEQ ID NO:15)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	6/6
56	(SEQ ID NO:18)-GG-(LHRH) ^a				
57	(Inv) ^b -GG-(SEQ ID NO:15)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	6/6
58	(Inv) ^b -GG-(SEQ ID NO:18)-GG-(LHRH) ^a				
59	(SEQ ID NO:19)-GG-(LHRH) ^a	100 µg/dose	6/6	25 µg/dose	14/14

Table 2 (Cont'd)

106	(SEQ ID NO:105)-GG-(LHRH) ^a	IFA (0,3,6wpi)		Alum (0,3 wpi)	
53	(SEQ ID NO:15)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	1/6
60	(Inv) ^b -(SEQ ID NO:15)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	4/6
61	(SEQ ID NO:20)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	4/6
62	(Inv) ^b -GG-(SEQ ID NO:20)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	2/6
63	(Inv) ^b -(SEQ ID NO:21)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	1/6
64	(SEQ ID NO:22)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	4/6

b. HBsAg Th Derivatives

SEQ ID NO:	Description of Antigenic Peptide	Formulations			
		no. castrated		no. castrated	
65	(SEQ ID NO:23)-GG-(LHRH) ^a	400 µg/dose IFA (0,3,6wpi)	10/10	400 µg/dose Alum (0,3,6 wpi)	0/5
66	(SEQ ID NO:26)-GG-(LHRH) ^a	400 µg/dose IFA (0,3,6wpi)	9/10	400 µg/dose Alum (0,3,6 wpi)	2/5
67	(SEQ ID NO:27)-GG-(LHRH) ^a				
68	(SEQ ID NO:28)-GG-(LHRH) ^a				
69	(SEQ ID NO:29)-GG-(LHRH) ^a				
70	(SEQ ID NO:30)-GG-(LHRH) ^a				
71	(SEQ ID NO:31)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	8/8
72	(SEQ ID NO:32)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	4/6
73	(SEQ ID NO:33)-GG-(LHRH) ^a	100 µg/dose IFA (0,3,6wpi)	4/6	25 µg/dose Alum (0,3 wpi)	0/6
74	(SEQ ID NO:34)-GG-(LHRH) ^a	100 µg/dose IFA (0,3,6wpi)	6/6	25 µg/dose Alum (0,3 wpi)	5/8
75	(Inv) ^b -GG-(SEQ ID NO:34)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	4/6
76	(SEQ ID NO:35)-GG-(LHRH) ^a	N.D.		25 µg/dose Alum (0,3 wpi)	0/8
	KLH ^c -(LHRH) ^a	N.D.		50 µg/dose Alum (0,3 wpi)	2/8

^a LHRH = EHWSYGLRPG (SEQ ID NO:77)
^b INV = Invasin domain (SEQ ID NO:78)
^c KLH = Keyhole limpet hemocyanin
^d Hinge spacer = PPXPXP (SEQ ID NO:79)

Table 3

Evaluation of Antibody Specificity
for the Target Antigenic Site

SEQ ID NO:	LHRH Reactivity ^a	Th Reactivity ^b
53 and 56	6/6	0/6 ^c
71	8/8	0/8 ^d
74	4/8	0/8 ^e

- ^a Number of animals with anti-LHRH titers
> 1:1000/total animals immunized. The ELISA peptide was SEQ ID NO:77.
- ^b Number of animals with anti-Th reactivity
> 0.100 A₄₉₀ /total animals immunized. Sera were diluted 1:100 and all A₄₉₀ values were at background values for the respective Th peptides.
- ^c ELISA peptide was a mixture of two peptides: SEQ ID NOS:15 and 18.
- ^d ELISA peptide was SEQ ID NO:31.
- ^e ELISA peptide was SEQ ID NO:34.

Table 4

Evaluation of Artificial Th/LHRH Peptide Compositions
Including Mixture

Immunogen ^a	0 wpi	5 wpi	8 wpi	10 wpi	14 wpi	18 wpi	22 wpi
SEQ ID NO:64 + Alum	0/8 ^b	3/8	8/8	8/8	7/8	5/8	3/8
SEQ ID NOS:57 and 58 + Alum	0/6	6/6	6/6	6/6	6/6	6/6	6/6
SEQ ID NO:64 + SEQ ID NOS:57 and 58 + Alum	0/6	6/6	6/6	6/6	6/6	6/6	6/6

^a Individual LHRH peptide compositions or the mixed LHRH peptide composition were formulated on alum. Immunization schedule: 25µg/dose at 0 and 3 wpi.

^b Number of animals immunocastrated/total number of animals in group. Animals were scored as immunocastrated when serum testosterone values were <0.1 nmol/L to undetectable.

Table 5 Immunogenicity of Somatostatin Antigenic Peptides

SEQ ID NO:	Description of Antigenic Peptide	Adjuvant	Immunogenicity		
			WPI ^b	Responding n=4	Log ₁₀ ELISA Titer ^c
80	Somatostatin ^a	0.4% Alum (0, 2, 4 WPI)	6 8	0 0	- -
81, 82, 83	(SEQ ID NOS: 6, 7, 8) -GG- (Somatostatin)	0.4% Alum (0, 3, 6 WPI)	5 8	1/4 4/4	2.38 3.33
84, 85, 86	(Somatostatin) -GG- (SEQ ID NOS: 6, 7, 8)	0.4% Alum (0, 3, 6 WPI)	5 8	3/4 4/4	3.24 3.12
87	(SEQ ID NO: 31) -GG- Somatostatin	0.4% Alum (0, 3, 6 WPI)	8	4/4	3.12

^a Sequence of somatostatin: AGCKNFFWKFTSC (SEQ ID NO: 80)

^b WPI = Weeks post-immunization

^c Test results for pooled sera from ELISA-reactive animals.

Table 6
Immunogenicity of IgE-CH3 Antigenic Peptides

SEQ ID NO:	Description of Antigenic Peptide	Immunogenicity	
		No. responding	Log ₁₀ ELISA ^b
93, 94	(SEQ ID NOS:15,18)-GG-(SEQ ID NO:92) ^a	3/3	3.30
95, 96, 97	(SEQ ID NOS:6,7,8)-GG-(SEQ ID NO:92) ^a	3/3	3.32
98	(SEQ ID NO:31)-GG-(SEQ ID NO:92) ^a	3/3	2.35
99	Inv ^c -GG-(SEQ ID NO:31)-GG-(SEQ ID NO:92) ^a	3/3	3.28
	KLH ^d -(SEQ ID NO:92) ^a	2/2	0.49

^a Modified IgE-CH3 site (SEQ ID NO:92)

^b Average for animals of the group

^c Invasin domain peptide (SEQ ID NO:78)

^d Keyhole Limpet Hemocyanin

Table 7
**Artificial Th and SSAL Target Antigenic Peptides for Improved Immunogenicity
 and Breadth of FMDV Neutralization**

SEQ ID NO:	Target Antigenic Peptide	WPI	No. of Animals Responding (n=3)	Log ₁₀ Anti-FMDV-Vp1 ^d ELISA Titer	Log ₁₀ of FMDV (MPD ₅₀) ^{d,e} Neutralized by Serum						
					A ₁₂ FP	O-1 (JH)	A-FL	O-1 P2	A-23	Asi a 1	A 1
101	(SEQ ID NO:31)-GG-O _{complete} SSAL[134-158 (T→C) ^a -169], cyclized	5	3	5.158	3.0	4.5	2.0				5.0
		10	3				2.5	4.5	6.0		
102	Inv-GG-(SEQ ID NO:31)-GG-Asia SSAL[134 (T→C) ^b -158 (R→C) ^c -169], cyclized	5	3	5.256	3.0	4.5	2.0				4.5
		10	3				1.0	3.0	2.5		

^a T₁₅₈ of the native sequence was replaced by C.

^b T₁₃₄ of native sequence was replaced by C.

^c R₁₅₈ of the native sequence was replaced by C.

^d Reactivities of pooled sera from ELISA-reactive animals.

^e Serum for neutralization assays diluted 1:100.

TABLE 8

CETP ANTIGENIC PEPTIDES

SEQ ID NO	DESCRIPTION OF ANTIGENIC PEPTIDE
110,111	(SEQ ID NOS:15,18)-□NLys-(SEQ ID NO:106)
112,113	(SEQ ID NOS:15,18)-□NLys-(SEQ ID NO:107)
114	(SEQ ID NO:31)-□NLys-(SEQ ID NO:107)
115	(SEQ ID NO:22)-□NLys-(SEQ ID NO:107)
116,117	(SEQ ID NO:15,18)-□NLys-(SEQ ID NO:108) ^a
118,119	(SEQ ID NO:15,18)-□NLys-(SEQ ID NO:109) ^b

Note:

^a = Phe Gly Phe Pro Lys His Leu Leu Val Asp Phe
Leu Gln Ser Leu Ser

(SEQ ID NO:108)

^b = Leu Asp Gly Cys Leu Leu Leu Gln Met Asp Phe
Gly Phe Pro Lys His Leu Leu Val Asp Phe Leu
Gln Ser Leu Ser

(SEQ ID NO:109)

Table 9

Amino Acid Sequence of HIV Neutralizing Epitopes (SEQ ID NO)	Description of Th Epitopes	Peptide Code	Amino Acid Sequences Of Representative Peptide Constructs
ESVEINCTRPNNNTRKSIRIGPGQAFYATGD (SEQ ID NO:125)	Simplified MVF Th lib (SEQ ID No:15,18)	p2846b	ISISEIKGVIVHKIEGILF-(cN)K-ESVEINCTRPNNNTRKSIRIGPGQAFYATGD T RT TR T (SEQ ID NO:126,127)
	Simplified [I,L] MVF Th (SEQ ID No:110,111)	p2868b	ISLTEIRTVIVTRLETVLF--(cN)K-ESVEINCTRPNNNTRKSIRIGPGQAFYATGD I I I (SEQ ID NO:128,129)
KSSGKLISL (SEQ ID NO:130)	Simplified MVF Th (SEQ ID No:15,18)	p2942	KSSGKLISL-(cN)K-ISISEIKGVIVHKIEGILF T RT TR T (SEQ ID NO:136,137)
	Simplified MVF Th (SEQ ID No:15,18)	p2937	CNGRLYCGP-(cN)K-ISISEIKGVIVHKIEGILF T RT TR T (SEQ ID NO:138,139)
(C)GTKLVCFAA (SEQ ID NO:132)	Simplified MVF Th (SEQ ID No:15,18)	p2939	(C)GTKLVCFAA-(cN)K-ISISEIKGVIVHKIEGILF T RT TR T (SEQ ID NO:140,141)
KRIVIGPQT (SEQ ID NO:133)	Simplified MVF Th (SEQ ID No:15,18)	p2944	KRIVIGPQT-(cN)K-ISISEIKGVIVHKIEGILF T RT TR T (SEQ ID NO:142,143)
CAGGLTCSV (SEQ ID NO:134)	Simplified MVF Th (SEQ ID No:15,18)	p2940	CAGGLTCSV-(cN)K-ISISEIKGVIVHKIEGILF T RT TR T (SEQ ID NO:144,145)
(C)SGRLYCHESW (SEQ ID NO:135)	Simplified MVF Th (SEQ ID No:15,18)	p2941	(C)SGRLYCHESW-(cN)K-ISISEIKGVIVHKIEGILF T RT TR T (SEQ ID NO:146,147)

Table 10
Immunogenicity of Representative Peptides of the Invention

Species	Group #	Adjuvant and Immunization Schedule	Peptide Code	Description of Representative Peptide Constructs	WPI	Log ₁₀ ELISA Titer on Target Antigen	Serum Titer by Neutralization Assay (HIV-1 MN/H9)	
							50% Inhibit.	90% Inhibit.
Guinea Pig	1	Oil in water emulsion (0, 3, 6 WPI)	p2846 b (Seq ID Nos. 148, 149)	IS(1,4,9 PALINDROMIC Th simplified lib)LF-GG-"V3" All Consensus	5	4.461	3972	425
					8	4.383	10276	535
					10	4.245	4245	1006
	2	Oil in water emulsion (0, 3, 6 WPI)	p2868 (Seq ID Nos. 150, 151)	lib(1, L) - (cN)K - "V3" All Consensus	5	4.489	1198	206
					8	4.291	3295	1092
					10	4.239	4233	1147
Baboon	1	Oil in water emulsion (0, 3, 6 WPI)	p2868 (Seq ID Nos. 150, 151)	lib(1, L) - (cN)K - "V3" All Consensus	8	4.375	948	152
					11	over	23745	2811

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: UNITED BIOMEDICAL INC., ET AL.
- (ii) TITLE OF INVENTION: ARTIFICIAL T HELPER CELL
EPITOPES AS IMMUNE STIMULATORS FOR SYNTHETIC
PEPTIDE IMMUNOGENS
- (iii) NUMBER OF SEQUENCES: 151
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Morgan & Finnegan, L.L.P.
 - (B) STREET: 345 Park Avenue
 - (C) CITY: New York
 - (D) STATE: NY
 - (E) COUNTRY: USA
 - (F) ZIP: 10154-0054
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC Windows
 - (D) SOFTWARE: Word 97
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: TO BE ASSIGNED
 - (B) FILING DATE: 21-JUNE-1999
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 09/100,412
 - (B) FILING DATE: 20-JUNE-1998
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Maria H. Lin
 - (B) REGISTRATION NUMBER: 29,323
 - (C) REFERENCE/DOCKET NUMBER: 1151-4158PC1
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 212-758-4800
 - (B) TELEFAX: 212-751-6849

(2) INFORMATION FOR SEQ ID NO:1:

- 66 -

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu
1 5 10
Glu Gly Val
15

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Leu Ser Asp Leu Lys Gly Leu Leu Leu His Lys
1 5 10
Leu Asp Gly Leu
15

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Ile Ser Glu Ile Arg Gly Ile Ile Ile His Arg
1 5 10
Ile Glu Gly Ile
15

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Asp Val Ser Asp Val Lys Gly Val Val Val His Lys
1          5          10
Val Asp Gly Val
      15

```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Phe Ser Asp Phe Lys Gly Phe Phe Phe His Lys
1 5 10
Phe Asp Gly Phe
15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```

Ile Ser Glu Ile Lys Gly Val Ile Val His Lys Ile
1          5          10
Glu Gly Ile
          15

```

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(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr	Arg	Met
1				5					10		
Glu	Thr	Met									
		15									

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His	Lys
1				5				10		
Leu	Glu	Gly	Val							
		15								

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Ile Thr Glu Ile Arg Thr Val Ile Val Thr Arg Ile
1 5 10
Glu Thr Ile
15

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Glu Ile Lys Gly Val Ile Val His Lys Leu
1 5 10
Glu Gly Met
15

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Leu Thr Glu Met Arg Thr Val Ile Val Thr Arg Met
1 5 10
Glu Thr Val
15

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ile Thr Glu Ile Arg Thr Val Ile Val Thr Arg Ile
1 5 10
Glu Thr Ile
15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Ser Glu Met Lys Gly Val Ile Val His Lys Met
1 5 10
Glu Gly Met
15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Leu Thr Glu Ile Arg Thr Val Ile Val Thr Arg Leu
1 5 10
Glu Thr Val
15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```

Ile Ser Ile Ser Glu Ile Lys Gly Val Ile Val His
1          5          10
Lys Ile Glu Gly Ile Leu Phe
          15

```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

Ile Ser Met Thr Glu Ile Arg Thr Val Ile Val Thr
1      5      10
Arg Met Glu Thr Met Leu Phe
      15

```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```

Ile Ser Leu Ser Glu Ile Lys Gly Val Ile Val His
1          5          10
Lys Leu Glu Gly Val Leu Phe
          15

```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr
1 5 10
Arg Ile Glu Thr Ile Leu Phe

15

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ile	Ser	Leu	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His
1				5				10			
Lys	Leu	Glu	Gly	Met	Leu	Phe					
				15							

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ile	Ser	Ile	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
1				5				10			
Arg	Ile	Glu	Thr	Ile	Leu	Phe					
				15							

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ile	Ser	Met	Ser	Glu	Met	Lys	Gly	Val	Ile	Val	His
1				5				10			
Lys	Met	Glu	Gly	Met	Leu	Phe					
				15							

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(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ile	Ser	Leu	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
1				5					10		
Arg	Leu	Glu	Thr	Val	Leu	Phe					
				15							

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Phe	Phe	Leu	Leu	Thr	Arg	Ile	Leu	Thr	Ile	Pro	Gln
1				5					10		
Ser	Leu	Asp									
				15							

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Lys	Lys	Lys	Phe	Phe	Leu	Leu	Thr	Arg	Ile	Leu	Thr
1				5					10		
Ile	Pro	Gln	Ser	Leu	Asp						
				15							

(2) INFORMATION FOR SEQ ID NO:25:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Phe	Phe	Leu	Leu	Thr	Arg	Ile	Leu	Thr	Ile	Pro	Gln
1				5				10			
Ser	Leu										

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Lys	Lys	Lys	Leu	Phe	Leu	Leu	Thr	Lys	Leu	Leu	Thr
1				5				10			
Leu	Pro	Gln	Ser	Leu	Asp						
			15								

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Arg	Arg	Arg	Ile	Lys	Ile	Ile	Thr	Arg	Ile	Ile	Thr
1				5				10			
Ile	Pro	Leu	Ser	Ile	Arg						
			15								

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Lys Lys Lys Val Arg Val Val Thr Lys Val Val Thr
1 5 10
Val Pro Ile Ser Val Asp
15

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Lys Lys Lys Phe Phe Phe Phe Thr Lys Phe Phe Thr
1 5 10
Phe Pro Val Ser Phe Asp
15

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Lys Lys Lys Leu Phe Leu Leu Thr Lys Leu Leu Thr
1 5 10
Leu Pro Phe Ser Leu Asp
15

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Lys	Lys	Lys	Ile	Ile	Thr	Ile	Thr	Arg	Ile	Ile	Thr
1				5					10		
Ile	Ile	Thr	Thr	Ile	Asp						
			15								

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Lys	Lys	Lys	Ile	Ile	Thr	Ile	Thr	Arg	Ile	Ile	Thr
1				5					10		
Ile	Ile	Thr	Thr	Ile							
			15								

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Lys	Lys	Lys	Met	Met	Thr	Met	Thr	Arg	Met	Ile	Thr
1				5					10		
Met	Ile	Thr	Thr	Ile	Asp						
			15								

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

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Phe Ile Thr Met Asp Thr Lys Phe Leu Leu Ala Ser
 1 5 10
 Thr His Ile Leu
 15

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Lys Lys Lys Phe Ile Thr Met Asp Thr Lys Phe Leu
 1 5 10
 Leu Ala Ser Thr His Ile Leu
 15

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu
 1 5 10
 Glu Gly Val Gly Gly Glu His Trp Ser Tyr Gly Leu
 15 20
 Arg Pro Gly
 25

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Asp Leu Ser Asp Leu Lys Gly Leu Leu Leu His Lys

(2) INFORMATION FOR SEQ ID NO:38:

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```

Asp Phe Ser Asp Phe Lys Gly Phe Phe Phe His Lys
1           5           10
Phe Asp Gly Phe Gly Gly Glu His Trp Ser Tyr Gly
          15           20
Leu Arg Pro Gly
25

```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```

Ile Ser Glu Ile Lys Gly Val Ile Val His Lys Ile
1           5           10
Glu Gly Ile Gly Gly Glu His Trp Ser Tyr Gly Leu
          15           20
Arg Pro Gly
25

```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```

Met Thr Glu Ile Arg Thr Val Ile Val Thr Arg Met
1           5           10
Glu Thr Met Gly Gly Glu His Trp Ser Tyr Gly Leu
          15           20
Arg Pro Gly
25

```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Leu	Ser	Glu	Ile	Lys	Gly	val	Ile	Val	His	Lys	Leu
1				5				10			
Glu	Gly	Val	Gly	Gly	Glu	His	Trp	Ser	Tyr	Gly	Leu
		15					20				
Arg	Pro	Gly									
25											

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Ile	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr	Arg	Ile
1				5				10			
Glu	Thr	Ile	Gly	Gly	Glu	His	Trp	Ser	Tyr	Gly	Leu
		15					20				
Arg	Pro	Gly									
25											

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 45 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Thr	Ala	Lys	Ser	Lys	Lys	Phe	Pro	Ser	Tyr	Thr	Ala
1				5					10		
Thr	Tyr	Gln	Phe	Gly	Gly	Ile	Ser	Glu	Ile	Lys	Gly
		15					20				
Val	Ile	Val	His	Lys	Ile	Glu	Gly	Ile	Gly	Gly	Glu
25				30						35	
His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly			
		40						45			

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(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Thr	Ala	Lys	Ser	Lys	Lys	Phe	Pro	Ser	Tyr	Thr	Ala
1				5					10		
Thr	Tyr	Gln	Phe	Gly	Gly	Ile	Thr	Glu	Ile	Arg	Thr
		15				20					
Val	Ile	Val	Thr	Arg	Ile	Glu	Thr	Ile	Gly	Gly	Glu
25				30					35		
His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly			
				40							

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Met	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His	Lys	Leu
1				5					10		
Glu	Gly	Met	Gly	Gly	Glu	His	Trp	Ser	Tyr	Gly	Leu
		15				20					
Arg	Pro	Gly									
25											

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

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```

Leu Thr Glu Met Arg Thr Val Ile Val Thr Arg Met
1           5           10
Glu Thr Val Gly Gly Glu His Trp Ser Tyr Gly Leu
          15           20
Arg Pro Gly
25

```

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

```

Ile Thr Glu Ile Arg Thr Val Ile Val Thr Arg Ile
1           5           10
Glu Thr Ile Gly Gly Glu His Trp Ser Tyr Gly Leu
          15           20
Arg Pro Gly
25

```

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```

Met Ser Glu Met Lys Gly Val Ile Val His Lys Met
1           5           10
Glu Gly Met Gly Gly Glu His Trp Ser Tyr Gly Leu
          15           20
Arg Pro Gly
25

```

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

```

Leu Thr Glu Ile Arg Thr Val Ile Val Thr Arg Leu
 1             5             10
Glu Thr Val Gly Gly Glu His Trp Ser Tyr Gly Leu
      15             20
Arg Pro Gly
25

```

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

```

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala
 1             5             10
Thr Tyr Gln Phe Gly Gly Leu Thr Glu Ile Arg Thr
      15             20
Val Ile Val Thr Arg Leu Glu Thr Val Gly Gly Glu
 25             30             35
His Trp Ser Tyr Gly Leu Arg Pro Gly
      40             45

```

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

```

Ile Ser Ile Ser Glu Ile Lys Gly Val Ile Val His
 1             5             10
Lys Ile Glu Gly Ile Leu Phe Gly Gly Glu His Trp
      15             20
Ser Tyr Gly Leu Arg Pro Gly
 25             30

```

(2) INFORMATION FOR SEQ ID NO:54:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Ile	Ser	Met	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
1				5				10			
Arg	Met	Glu	Thr	Met	Leu	Phe	Gly	Gly	Glu	His	Trp
		15					20				
Ser	Tyr	Gly	Leu	Arg	Pro	Gly					
25				30							

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Ile	Ser	Leu	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His
1				5				10			
Lys	Leu	Glu	Gly	Val	Leu	Phe	Gly	Gly	Glu	His	Trp
		15					20				
Ser	Tyr	Gly	Leu	Arg	Pro	Gly					
25				30							

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Ile	Ser	Ile	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
1				5				10			
Arg	Ile	Glu	Thr	Ile	Leu	Phe	Gly	Gly	Glu	His	Trp
		15					20				
Ser	Tyr	Gly	Leu	Arg	Pro	Gly					
25				30							

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 49 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

```

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala
1          5          10
Thr Tyr Gln Phe Gly Gly Ile Ser Ile Ser Glu Ile
          15          20
Lys Gly Val Ile Val His Lys Ile Glu Gly Ile Leu
25          30          35
Phe Gly Gly Glu His Trp Ser Tyr Gly Leu Arg Pro
          40          45
Gly

```

(2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 49 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala
1          5          10
Thr Tyr Gln Phe Gly Gly Ile Ser Ile Thr Glu Ile
          15          20
Arg Thr Val Ile Val Thr Arg Ile Glu Thr Ile Leu
25          30          35
Phe Gly Gly Glu His Trp Ser Tyr Gly Leu Arg Pro
          40          45
Gly

```

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

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```

Ile Ser Leu Ser Glu Ile Lys Gly Val Ile Val His
1           5           10
Lys Leu Glu Gly Met Leu Phe Gly Gly Glu His Trp
          15           20
Ser Tyr Gly Leu Arg Pro Gly
25           30

```

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 47 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala
1           5           10
Thr Tyr Gln Phe Ile Ser Ile Ser Glu Ile Lys Gly
          15           20
Val Ile Val His Lys Ile Glu Gly Ile Leu Phe Gly
25           30           35
Gly Glu His Trp Ser Tyr Gly Leu Arg Pro Gly
          40           45

```

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

```

Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr
1           5           10
Arg Ile Glu Thr Ile Leu Phe Gly Gly Glu His Trp
          15           20
Ser Tyr Gly Leu Arg Pro Gly
25           30

```

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 49 amino acids
 (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Thr	Ala	Lys	Ser	Lys	Lys	Phe	Pro	Ser	Tyr	Thr	Ala
1				5					10		
Thr	Tyr	Gln	Phe	Gly	Gly	Ile	Ser	Ile	Thr	Glu	Ile
		15				20					
Arg	Thr	Val	Ile	Val	Thr	Arg	Ile	Glu	Thr	Ile	Leu
25					30					35	
Phe	Gly	Gly	Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro
			40					45			
Gly											

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Thr	Ala	Lys	Ser	Lys	Lys	Phe	Pro	Ser	Tyr	Thr	Ala
1				5					10		
Thr	Tyr	Gln	Phe	Ile	Ser	Met	Ser	Glu	Met	Lys	Gly
		15				20					
Val	Ile	Val	His	Lys	Met	Glu	Gly	Met	Leu	Phe	Gly
25					30					35	
Gly	Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly	
			40					45			

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Ile	Ser	Leu	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
1				5					10		
Arg	Leu	Glu	Thr	Val	Leu	Phe	Gly	Gly	Glu	His	Trp
		15				20					
Ser	Tyr	Gly	Leu	Arg	Pro	Gly					

25

30

(2) INFORMATION FOR SEQ ID NO:65

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65

Phe	Phe	Leu	Leu	Thr	Arg	Ile	Leu	Thr	Ile	Pro	Gln
1				5				10			
Ser	Leu	Asp	Gly	Gly	Glu	His	Trp	Ser	Tyr	Gly	Leu
		15					20				
Arg	Pro	Gly									
25											

(2) INFORMATION FOR SEQ ID NO:66

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66

Lys	Lys	Lys	Leu	Phe	Leu	Leu	Thr	Lys	Leu	Leu	Thr
1				5				10			
Leu	Pro	Gln	Ser	Leu	Asp	Gly	Gly	Glu	His	Trp	Ser
		15					20				
Tyr	Gly	Leu	Arg	Pro	Gly						
25				30							

(2) INFORMATION FOR SEQ ID NO:67

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67

Arg	Arg	Arg	Ile	Lys	Ile	Ile	Thr	Arg	Ile	Ile	Thr
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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```

1           5           10
Ile Pro Leu Ser Ile Arg Gly Gly Glu His Trp Ser
           15           20
Tyr Gly Leu Arg Pro Gly
25           30

```

(2) INFORMATION FOR SEQ ID NO:68

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68

```

Lys Lys Lys Val Arg Val Val Thr Lys Val Val Thr
1           5           10
Val Pro Ile Ser Val Asp Gly Gly Glu His Trp Ser
           15           20
Tyr Gly Leu Arg Pro Gly
25

```

(2) INFORMATION FOR SEQ ID NO:69

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69

```

Lys Lys Lys Phe Phe Phe Phe Thr Lys Phe Phe Thr
1           5           10
Phe Pro Val Ser Phe Asp Gly Gly Glu His Trp Ser
           15           20
Tyr Gly Leu Arg Pro Gly
25           30

```

(2) INFORMATION FOR SEQ ID NO:70

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70

```

Lys Lys Lys Leu Phe Leu Leu Thr Lys Leu Leu Thr
 1              5              10
Leu Pro Phe Ser Leu Asp Gly Gly Glu His Trp Ser
      15              20
Tyr Gly Leu Arg Pro Gly
25              30

```

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

```

Lys Lys Lys Ile Ile Thr Ile Thr Arg Ile Ile Thr
 1              5              10
Ile Ile Thr Thr Ile Asp Gly Gly Glu His Trp Ser
      15              20
Tyr Gly Leu Arg Pro Gly
25              30

```

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

```

Lys Lys Lys Ile Ile Thr Ile Thr Arg Ile Ile Thr
 1              5              10
Ile Ile Thr Thr Ile Gly Gly Glu His Trp Ser Tyr
      15              20
Gly Leu Arg Pro Gly
25

```

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Lys	Lys	Lys	Met	Met	Thr	Met	Thr	Arg	Met	Ile	Thr
1				5					10		
Met	Ile	Thr	Thr	Ile	Asp	Gly	Gly	Glu	His	Trp	Ser
		15					20				
Tyr	Gly	Leu	Arg	Pro	Gly						
25					30						

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Phe	Ile	Thr	Met	Asp	Thr	Lys	Phe	Leu	Leu	Ala	Ser
1				5					10		
Thr	His	Ile	Leu	Gly	Gly	Glu	His	Trp	Ser	Tyr	Gly
		15					20				
Leu	Arg	Pro	Gly								
25											

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Thr	Ala	Lys	Ser	Lys	Lys	Phe	Pro	Ser	Tyr	Thr	Ala
1				5					10		
Thr	Tyr	Gln	Phe	Gly	Gly	Phe	Ile	Thr	Met	Asp	Thr
		15					20				
Lys	Phe	Leu	Leu	Ala	Ser	Thr	His	Ile	Leu	Gly	Gly
25					30					35	
Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly		
			40					45			

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(2) INFORMATION FOR SEQ ID NO:76:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Lys	Lys	Lys	Phe	Ile	Thr	Met	Asp	Thr	Lys	Phe	Leu
1				5					10		
Leu	Ala	Ser	Thr	His	Ile	Leu	Gly	Gly	Glu	His	Trp
		15					20				
Ser	Tyr	Gly	Leu	Arg	Pro	Gly					
25					30						

(2) INFORMATION FOR SEQ ID NO:77:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly
1				5					10

(2) INFORMATION FOR SEQ ID NO:78:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Thr	Ala	Lys	Ser	Lys	Lys	Phe	Pro	Ser	Tyr	Thr	Ala
1				5					10		
Thr	Tyr	Gln	Phe								
		15									

(2) INFORMATION FOR SEQ ID NO:79:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

```

Pro Pro Xaa Pro Xaa Pro
1              5

```

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

```

Ala Gly Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr
1              5              10
Ser Cys

```

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

```

Ile Ser Glu Ile Lys Gly Val Ile Val His Lys Ile
1              5              10
Glu Gly Ile Gly Gly Ala Gly Cys Lys Asn Phe Phe
          15              20
Trp Lys Thr Phe Thr Ser Cys
25              30

```

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Met	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr	Arg	Met
1				5					10		
Glu	Thr	Met	Gly	Gly	Ala	Gly	Cys	Lys	Asn	Phe	Phe
		15					20				
Trp	Lys	Thr	Phe	Thr	Ser	Cys					
25					30						

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Leu	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His	Lys	Leu
1				5					10		
Glu	Gly	Val	Gly	Gly	Ala	Gly	Cys	Lys	Asn	Phe	Phe
		15					20				
Trp	Lys	Thr	Phe	Thr	Ser	Cys					
25					30						

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Ala	Gly	Cys	Lys	Asn	Phe	Phe	Trp	Lys	Thr	Phe	Thr
1				5					10		
Ser	Cys	Gly	Gly	Ile	Ser	Glu	Ile	Lys	Gly	Val	Ile
		15					20				
Val	His	Lys	Ile	Glu	Gly	Ile					
25					30						

(2) INFORMATION FOR SEQ ID NO:85:

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Ala	Gly	Cys	Lys	Asn	Phe	Phe	Trp	Lys	Thr	Phe	Thr
1				5					10		
Ser	Cys	Gly	Gly	Met	Thr	Glu	Ile	Arg	Thr	Val	Ile
		15					20				
Val	Thr	Arg	Met	Gly	Thr	Met					
25					30						

(2) INFORMATION FOR SEQ ID NO:86:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Ala	Gly	Cys	Lys	Asn	Phe	Phe	Trp	Lys	Thr	Phe	Thr
1				5					10		
Ser	Cys	Gly	Gly	Leu	Ser	Glu	Ile	Lys	Gly	Val	Ile
		15					20				
Val	His	Lys	Leu	Glu	Gly	Val					
25					30						

(2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 34 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Lys	Lys	Lys	Ile	Ile	Thr	Ile	Thr	Arg	Ile	Ile	Thr
1				5					10		
Ile	Ile	Thr	Thr	Ile	Asp	Gly	Gly	Ala	Gly	Cys	Lys
		15					20				
Asn	Phe	Phe	Trp	Lys	Thr	Phe	Thr	Ser	Cys		
25					30						

(2) INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Cys	Asn	Gln	Gly	Ser	Phe	Leu	Thr	Lys	Gly	Pro	Ser
1				5					10		
Lys	Leu	Asn	Asp	Arg	Ala	Asp	Ser	Arg	Arg	Ser	Leu
		15					20				
Trp	Asp	Gln	Gly	Asn	Cys						
25				30							

(2) INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 47 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Ile	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His	Lys	Ile
1				5					10		
Glu	Gly	Ile	Gly	Gly	Cys	Asn	Gln	Gly	Ser	Phe	Leu
		15					20				
Thr	Lys	Gly	Pro	Ser	Lys	Leu	Asn	Asp	Arg	Ala	Asp
25				30					35		
Ser	Arg	Arg	Ser	Leu	Trp	Asp	Gln	Gly	Asn	Cys	
			40					45			

(2) INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 47 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

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```

Met Thr Glu Ile Arg Thr Val Ile Val Thr Arg Met
1           5           10
Glu Thr Met Gly Gly Cys Asn Gln Gly Ser Phe Leu
          15           20
Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Ala Asp
25           30           35
Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Cys
          40           45

```

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

```

Leu Ser Glu Ile Lys Gly Val Ile Val His Lys Leu
1           5           10
Glu Gly Val Gly Gly Cys Asn Gln Gly Ser Phe Leu
          15           20
Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Ala Asp
25           30           35
Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Cys
          40           45

```

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

```

Cys Gly Glu Thr Tyr Gln Ser Arg Val Thr His Pro
1           5           10
His Leu Pro Arg Ala Leu Met Arg Ser Thr Thr Lys
          15           20
Cys
25

```

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids

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(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

```

Ile Ser Ile Ser Glu Ile Lys Gly Val Ile Val His
1           5           10
Lys Ile Glu Gly Ile Leu Phe Gly Gly Cys Gly Glu
          15           20
Thr Tyr Gln Ser Arg Val Thr His Pro His Leu Pro
25           30           35
Arg Ala Leu Met Arg Ser Thr Thr Lys Cys
          40           45

```

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

```

Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr
1           5           10
Arg Ile Glu Thr Ile Leu Phe Gly Gly Cys Gly Glu
          15           20
Thr Tyr Gln Ser Arg Val Thr His Pro His Leu Pro
25           30           35
Arg Ala Leu Met Arg Ser Thr Thr Lys Cys
          40           45

```

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

```

Ile Ser Glu Ile Lys Gly Val Ile Val His Lys Ile
1           5           10
Glu Gly Ile Gly Gly Cys Gly Glu Thr Tyr Gln Ser
          15           20

```


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Arg Val Thr His Pro His Leu Pro Arg Ala Leu Met
 25 30 35
 Arg Ser Thr Thr Lys Cys
 40

(2) INFORMATION FOR SEQ ID NO:96:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 42 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

Met Thr Glu Ile Arg Thr Val Ile Val Thr Arg Met
 1 5 10
 Glu Thr Met Gly Gly Cys Gly Glu Thr Tyr Gln Ser
 15 20
 Arg Val Thr His Pro His Leu Pro Arg Ala Leu Met
 25 30 35
 Arg Ser Thr Thr Lys Cys
 40

(2) INFORMATION FOR SEQ ID NO:97:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 42 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

Leu Ser Glu Ile Lys Gly Val Ile Val His Lys Leu
 1 5 10
 Glu Gly Val Gly Gly Cys Gly Glu Thr Tyr Gln Ser
 15 20
 Arg Val Thr His Pro His Leu Pro Arg Ala Leu Met
 25 30 35
 Arg Ser Thr Thr Lys Cys
 40

(2) INFORMATION FOR SEQ ID NO:98:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 45 amino acids
 (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

Lys	Lys	Lys	Ile	Ile	Thr	Ile	Thr	Arg	Ile	Ile	Thr
1				5					10		
Ile	Ile	Thr	Thr	Ile	Asp	Gly	Gly	Cys	Gly	Glu	Thr
		15				20					
Tyr	Gln	Ser	Arg	Val	Thr	His	Pro	His	Leu	Pro	Arg
25				30					35		
Ala	Leu	Met	Arg	Ser	Thr	Thr	Lys	Cys			
		40						45			

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 63 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

Thr	Ala	Lys	Ser	Lys	Lys	Phe	Pro	Ser	Tyr	Thr	Ala
1				5					10		
Thr	Tyr	Gln	Phe	Gly	Gly	Lys	Lys	Lys	Ile	Ile	Thr
		15				20					
Ile	Thr	Arg	Ile	Ile	Thr	Ile	Ile	Thr	Thr	Ile	Asp
25				30					35		
Gly	Gly	Cys	Gly	Glu	Thr	Tyr	Gln	Ser	Arg	Val	Thr
		40				45					
His	Pro	His	Leu	Pro	Arg	Ala	Leu	Met	Arg	Ser	Thr
	50				55					60	
Thr	Lys	Cys									

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

Arg	His	Lys	Gln	Lys	Ile	Val	Ala	Pro	Val	Lys	Gln
1				5					10		

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```

Thr Leu Pro Pro Ser Val Pro Asn Leu Arg Gly Asp
      15                20
Leu Gln Val Leu Ala Gln Lys Val Ala Arg Thr Pro
25                      30          35
Cys Gly

```

(2) INFORMATION FOR SEQ ID NO:101:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 56 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

```

Lys Lys Lys Ile Ile Thr Ile Thr Arg Ile Ile Thr
1                5                10
Ile Ile Thr Thr Ile Asp Gly Gly Cys Lys Tyr Gly
      15                20
Glu Asn Ala Val Thr Asn Val Arg Gly Asp Leu Gln
25                      30          35
Val Leu Ala Gln Lys Ala Ala Arg Cys Leu Pro Thr
      40                45
Ser Phe Asn Tyr Gly Ala Ile Lys
      50                55

```

(2) INFORMATION FOR SEQ ID NO:102

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 72 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102

```

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala
1                5                10
Thr Tyr Gln Phe Gly Gly Lys Lys Lys Ile Ile Thr
      15                20
Ile Thr Arg Ile Ile Thr Ile Ile Thr Thr Ile Asp
25                      30          35
Gly Gly Cys Thr Tyr Gly Thr Gln Pro Ser Arg Arg
      40                45
Gly Asp Met Ala Ala Leu Ala Gln Arg Leu Ser Arg
      50                55          60
Cys Leu Pro Thr Ser Phe Asn Tyr Gly Ala Val Lys
      65                70

```

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(2) INFORMATION FOR SEQ ID NO:103

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103

Asn Ala Asn Pro

1

(2) INFORMATION FOR SEQ ID NO:104

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104

Ile	Ser	Ile	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His
1				5				10			
Lys	Ile	Glu	Gly	Ile	Leu	Phe	Lys	Asn	Ala	Asn	Pro
		15					20				
Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro
25				30						35	

(2) INFORMATION FOR SEQ ID NO:105

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105

Ile	Ser	Ile	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
1				5				10			
Arg	Ile	Glu	Thr	Ile	Leu	Phe	Lys	Asn	Ala	Asn	Pro
		15					20				
Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro
25				30						35	

[illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible]

Figure 1. The effect of the number of iterations (n) on the accuracy of the proposed algorithm. The figure shows two plots side-by-side. The left plot shows the error norm $\|e\|_2$ versus n , and the right plot shows the relative error $|e|/|y|$ versus n . Both plots show a decreasing trend as n increases from 0 to 1000. The left plot has a logarithmic y-axis ranging from 10^{-1} to 10^{-6} . The right plot has a linear y-axis ranging from 0 to 0.0008. Both plots have an x-axis ranging from 0 to 1000.

[illegible]

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(2) INFORMATION FOR SEQ ID NO:109

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109

Leu	Asp	Gly	Cys	Leu	Leu	Leu	Gln	Met	Asp	Phe	Gly
1				5					10		
Phe	Pro	Lys	His	Leu	Leu	Val	Asp	Phe	Leu	Gln	Ser
		15					20				
Leu	Ser										
25											

(2) INFORMATION FOR SEQ ID NO:110

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 46 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110

Ile	Ser	Ile	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His
1				5					10		
Lys	Ile	Glu	Gly	Ile	Leu	Phe	Lys	Arg	Asp	Gly	Phe
		15					20				
Leu	Leu	Leu	Gln	Met	Asp	Phe	Gly	Phe	Pro	Glu	His
25				30						35	
Leu	Leu	Val	Asp	Phe	Leu	Gln	Ser	Leu	Ser		
		40					45				

(2) INFORMATION FOR SEQ ID NO:111

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 46 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111

Ile	Ser	Ile	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

(2) INFORMATION FOR SEQ ID NO:112

(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112

(2) INFORMATION FOR SEQ ID NO:113

(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113

(2) INFORMATION FOR SEQ ID NO:114

(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114

Lys	Lys	Lys	Ile	Ile	Thr	Ile	Thr	Arg	Ile	Ile	Thr
1				5					10		
Ile	Ile	Thr	Thr	Ile	Asp	Lys	Phe	Gly	Phe	Pro	Glu
		15					20				
His	Leu	Leu	Val	Asp	Phe	Leu	Gln	Ser	Leu	Ser	
25				30						35	

(2) INFORMATION FOR SEQ ID NO:115

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115

Ile	Ser	Leu	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
1				5					10		
Arg	Leu	Glu	Thr	Val	Leu	Phe	Lys	Phe	Gly	Phe	Pro
		15					20				
Glu	His	Leu	Leu	Val	Asp	Phe	Leu	Gln	Ser	Leu	Ser
25				30						35	

(2) INFORMATION FOR SEQ ID NO:116

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116

Ile	Ser	Ile	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His
1				5					10		
Lys	Ile	Glu	Gly	Ile	Leu	Phe	Lys	Phe	Gly	Phe	Pro
		15					20				
Lys	His	Leu	Leu	Val	Asp	Phe	Leu	Gln	Ser	Leu	Ser
25				30						35	

(2) INFORMATION FOR SEQ ID NO:117

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 36 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117

```

Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr
1           5           10
Arg Ile Glu Thr Ile Leu Phe Lys Phe Gly Phe Pro
          15           20
Lys His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser
25           30           35

```

(2) INFORMATION FOR SEQ ID NO:118

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 46 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118

```

Ile Ser Ile Ser Glu Ile Lys Gly Val Ile Val His
1           5           10

Lys Ile Glu Gly Ile Leu Phe Lys Leu Asp Gly Cys
          15           20
Leu Leu Leu Gln Met Asp Phe Gly Phe Pro Lys His
25           30           35
Leu Leu Val Asp Phe Leu Gln Ser Leu Ser
          40           45

```

(2) INFORMATION FOR SEQ ID NO:119

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 46 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119

```

Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr
1           5           10
Arg Ile Glu Thr Ile Leu Phe Lys Leu Asp Gly Cys

```

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	15		20								
Leu	Leu	Leu	Gln	Met	Asp	Phe	Gly	Phe	Pro	Lys	His
25					30					35	
Leu	Leu	Val	Asp	Phe	Leu	Gln	Ser	Leu	Ser		
			40					45			

(2) INFORMATION FOR SEQ ID NO:120:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

Ile	Ser	Ile	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His
1				5				10			
Lys	Ile	Glu	Gly	Ile	Leu	Phe	Pro	Pro	Xaa	Pro	Xaa
		15				20					
Pro	Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly	
25				30				35			

(2) INFORMATION FOR SEQ ID NO:121:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

Ile	Ser	Ile	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
1				5				10			
Arg	Ile	Glu	Thr	Ile	Leu	Phe	Pro	Pro	Xaa	Pro	Xaa
		15				20					
Pro	Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly	
25				30				35			

(2) INFORMATION FOR SEQ ID NO:122:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 34 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

Lys Lys Lys Ile Ile Thr Ile Thr Arg Ile Ile Thr
1 5 10
Ile Ile Thr Thr Ile Asp Pro Pro Xaa Pro Xaa Pro
15 20
Glu His Trp Ser Tyr Gly Leu Arg Pro Gly
25 30

(2) INFORMATION FOR SEQ ID NO:123:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

Ile Ser Leu Thr Glu Ile Arg Thr Val Ile Val Thr
1 5 10
Arg Leu Glu Thr Val Leu Phe
15

(2) INFORMATION FOR SEQ ID NO:124:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr
1 5 10
Arg Ile Glu Thr Ile Leu Phe
15

(2) INFORMATION FOR SEQ ID NO:125:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

Glu	Ser	Val	Glu	Ile	Asn	Cys	Thr	Arg	Pro	Asn	Asn
1				5					10		
Asn	Thr	Arg	Lys	Ser	Ile	Arg	Ile	Gly	Pro	Gly	Gln
		15					20				
Ala	Phe	Tyr	Ala	Thr	Gly	Asp					
25					30						

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified site
- (B) LOCATION: 22
- (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

Ile	Ser	Ile	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His
1				5				10			
Lys	Ile	Glu	Gly	Ile	Leu	Phe	Xaa	Glu	Ser	Val	Glu
		15					20				
Ile	Asn	Cys	Thr	Arg	Pro	Asn	Asn	Asn	Thr	Arg	Lys
25				30					35		
Ser	Ile	Arg	Ile	Gly	Pro	Gly	Gln	Ala	Phe	Tyr	Ala
			40					48			
Thr	Gly	Asp									
		50									

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified site
- (B) LOCATION: 22
- (C) OTHER INFORMATION: /note= "(e-N)Lys"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

```

Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr
1           5           10
Arg Ile Glu Thr Ile Leu Phe Xaa Glu Ser Val Glu
15           20
Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys
25           30           35
Ser Ile Arg Ile Gly Pro Gly Gln Ala Phe Tyr Ala
40           48
Thr Gly Asp
50

```

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified site
- (B) LOCATION: 20
- (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

```

Ile Ser Leu Thr Glu Ile Arg Thr Val Ile Val Thr
1           5           10
Arg Leu Glu Thr Val Leu Phe Xaa Glu Ser Val Glu
15           20
Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys
25           30           35
Ser Ile Arg Ile Gly Pro Gly Gln Ala Phe Tyr Ala
40           48
Thr Gly Asp
50

```

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified site

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(B) LOCATION: 20

(C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

Ile	Ser	Ile	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr
1				5					10		
Arg	Ile	Glu	Thr	Val	Ile	Phe	Xaa	Glu	Ser	Val	Glu
		15					20				
Ile	Asn	Cys	Thr	Arg	Pro	Asn	Asn	Asn	Thr	Arg	Lys
25					30					35	
Ser	Ile	Arg	Ile	Gly	Pro	Gly	Gln	Ala	Phe	Tyr	Ala
			40					48			
Thr	Gly	Asp									
	50										

(2) INFORMATION FOR SEQ ID NO:130:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

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(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

Lys Ser Ser Gly Lys Leu Ile Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:131:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

Cys Asn Gly Arg Leu Tyr Cys Gly Pro
1 5

(2) INFORMATION FOR SEQ ID NO:132:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

Cys Gly Thr Lys Leu Val Cys Phe Ala Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:133:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

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Lys Arg Ile Val Ile Gly Pro Gln Thr
1 5

(2) INFORMATION FOR SEQ ID NO:134:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

Cys Ala Gly Gly Leu Thr Cys Ser Val
1 5

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

Cys Ser Gly Arg Leu Tyr Cys His Glu Ser Trp
1 5 10

(2) INFORMATION FOR SEQ ID NO:136:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified site
- (B) LOCATION: 10
- (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

Lys Ser Ser Gly Lys Leu Ile Ser Leu Xaa Ile Ser
1 5 10
Ile Ser Glu Ile Lys Gly Val Ile Val His Lys Ile

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15 20
 Glu Gly Ile Leu Phe
 25

(2) INFORMATION FOR SEQ ID NO:137:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
 (A) NAME/KEY: Modified site
 (B) LOCATION: 10
 (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

Lys Ser Ser Gly Lys Leu Ile Ser Leu Xaa Ile Ser
 1 5 10
 Ile Thr Glu Ile Arg Thr Val Ile Val Thr Arg Ile
 15 20
 Glu Thr Ile Leu Phe
 25

(2) INFORMATION FOR SEQ ID NO:138:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
 (A) NAME/KEY: Modified site
 (B) LOCATION: 10
 (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

Cys Asn Gly Arg Leu Tyr Cys Gly Pro Xaa Ile Ser
 1 5 10
 Ile Ser Glu Ile Lys Gly Val Ile Val His Lys Ile
 15 20
 Glu Gly Ile Leu Phe
 25

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(2) INFORMATION FOR SEQ ID NO:139:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
 (A) NAME/KEY: Modified site
 (B) LOCATION: 10
 (C) OTHER INFORMATION: /note= "(e-N)Lys"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

Cys Asn Gly Arg Leu Tyr Cys Gly Pro Xaa Ile Ser
1 5 10
Ile Thr Glu Ile Arg Thr Val Ile Val Thr Arg Ile
 15 20
Glu Thr Ile Leu Phe
25

(2) INFORMATION FOR SEQ ID NO:140:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
 (A) NAME/KEY: Modified site
 (B) LOCATION: 11
 (C) OTHER INFORMATION: /note= "(e-N)Lys"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

Cys Gly Thr Lys Leu Val Cys Phe Ala Ala Xaa Ile
1 5 10
Ser Ile Ser Glu Ile Lys Gly Val Ile Val His Lys
 15 20
Ile Glu Gly Ile Leu Phe
25 30

(2) INFORMATION FOR SEQ ID NO:141:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified site

(B) LOCATION: 11

(C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

Cys	Gly	Thr	Lys	Leu	Val	Cys	Phe	Ala	Ala	Xaa	Ile
1				5					10		
Ser	Ile	Thr	Glu	Ile	Arg	Thr	Val	Ile	Val	Thr	Arg
		15					20				
Ile	Glu	Thr	Ile	Leu	Phe						
25				30							

(2) INFORMATION FOR SEQ ID NO:142:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified site

(B) LOCATION: 10

(C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

Lys	Arg	Ile	Val	Ile	Gly	Pro	Gln	Thr	Xaa	Ile	Ser
1				5					10		
Ile	Ser	Glu	Ile	Lys	Gly	Val	Ile	Val	His	Lys	Ile
		15					20				
Glu	Gly	Ile	Leu	Phe							
25											

(2) INFORMATION FOR SEQ ID NO:143:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

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- (A) NAME/KEY: Modified site
- (B) LOCATION: 10
- (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

```
Lys Arg Ile Val Ile Gly Pro Gln Thr Xaa Ile Ser
1           5           10
Ile Thr Glu Ile Arg Thr Val Ile Val Thr Arg Ile
          15           20
Glu Thr Ile Leu Phe
25
```

(2) INFORMATION FOR SEQ ID NO:144:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified site
- (B) LOCATION: 10
- (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

```
Cys Ala Gly Gly Leu Thr Cys Ser Val Xaa Ile Ser
1           5           10
Ile Ser Glu Ile Lys Gly Val Ile Val His Lys Ile
          15           20
Glu Gly Ile Leu Phe
25
```

(2) INFORMATION FOR SEQ ID NO:145:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified site
- (B) LOCATION: 10
- (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

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```

Cys Ala Gly Gly Leu Thr Cys Ser Val Xaa Ile Ser
1           5           10
Ile Thr Glu Ile Arg Thr Val Ile Val Thr Arg Ile
          15           20
Glu Thr Ile Leu Phe
25

```

(2) INFORMATION FOR SEQ ID NO:146:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified site
- (B) LOCATION: 12
- (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

```

Cys Ser Gly Arg Leu Tyr Cys His Glu Ser Trp Xaa
1           5           10
Ile Ser Ile Ser Glu Ile Lys Gly Val Ile Val His
          15           20
Lys Ile Glu Gly Ile Leu Phe
25           30

```

(2) INFORMATION FOR SEQ ID NO:147:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified site
- (B) LOCATION: 12
- (C) OTHER INFORMATION: /note= "(e-N)Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

```

Cys Ser Gly Arg Leu Tyr Cys His Glu Ser Trp Xaa
1           5           10
Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr

```

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```

          15                20
Arg Ile Glu Gly Ile Leu Phe
25                30

```

(2) INFORMATION FOR SEQ ID NO:148:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 52 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

```

Ile Ser Ile Ser Glu Ile Lys Gly Val Ile Val His
1          5          10
Lys Ile Glu Gly Ile Leu Phe Gly Gly Glu Ser Val
          15          20
Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg
25          30          36
Lys Ser Ile Arg Ile Gly Pro Gly Gln Ala Phe Tyr
          40          45
Ala Thr Gly Asp
          50

```

(2) INFORMATION FOR SEQ ID NO:149:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 52 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

```

Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr
1          5          10
Arg Ile Glu Thr Ile Leu Phe Gly Gly Glu Ser Val
          15          20
Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg
25          30          36
Lys Ser Ile Arg Ile Gly Pro Gly Gln Ala Phe Tyr
          40          45
Ala Thr Gly Asp
          50

```

(2) INFORMATION FOR SEQ ID NO:150:

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 50 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
 (A) NAME/KEY: Modified site
 (B) LOCATION: 20
 (C) OTHER INFORMATION: /note= "(e-N)Lys"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

```

Ile Ser Leu Thr Glu Ile Arg Thr Val Ile Val Thr
1           5           10
Arg Leu Glu Thr Val Leu Phe Xaa Glu Ser Val Glu
          15           20
Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys
25           30           35
Ser Ile Arg Ile Gly Pro Gly Gln Ala Phe Tyr Ala
          40           48
Thr Gly
          50

```

(2) INFORMATION FOR SEQ ID NO:151:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 50 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
 (A) NAME/KEY: Modified site
 (B) LOCATION: 20
 (C) OTHER INFORMATION: /note= "(e-N)Lys"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

```

Ile Ser Ile Thr Glu Ile Arg Thr Val Ile Val Thr
1           5           10
Arg Ile Glu Thr Val Ile Phe Xaa Glu Ser Val Glu
          15           20
Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys
25           30           35
Ser Ile Arg Ile Gly Pro Gly Gln Ala Phe Tyr Ala
          40           48
Thr Gly
          50

```

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CLAIMS

We claim:

1. A T helper cell epitope selected from the group consisting of SEQ ID NOS: 6-22, 105, 123, 124, 31-35.

5

2. A T helper cell epitope according to Claim 1 for preparing a peptide immunogen represented by the formula

$$(A)_n-(\text{Target antigenic site})-(B)_o-(Th)_m-X$$

10

or

$$(A)_n-(Th)_m-(B)_o-(\text{Target antigenic site})-X$$

or

$$(A)_n--(B)_o(Th)_m-(B)_o-(\text{Target antigenic site})-X$$

or

15

$$(\text{Target antigenic site})-(B)_o-(Th)_m-(A)_n-X$$

or

$$(Th)_m-(B)_o-(\text{Target antigenic site})-(A)_n-X$$

wherein:

- A is an amino acid or a general immunostimulatory sequence, where n is more than one, the individual A's may be the same or different;

B is selected from the group consisting of amino acids, -HCH(X)CH₂SCH₂CO-, -NHCH(X)CH₂SCH₂CO(□-N)Lys-, -NHCH(X)CH₂S-succinimidyl(□-N)Lys-, and -NHCH(X)CH₂S-(succinimidyl)-;

- Th is an artificial helper T cell epitope selected from the group consisting of SEQ ID NOS:6-22, 105, 31-35 or an analog thereof;

"Target antigenic site" is a B cell epitope, a peptide hapten, or a immunologically reactive analog thereof;

- X is amino acid α-COOH or CONH₂ ;

n is from 1 to about 10;

m is from 1 to about 4; and

o is from 0 to about 10.

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3. A peptide immunogen according to claim 2 wherein the immunostimulatory sequence is SEQ ID NO:78.
- 5 4. A peptide immunogen according to Claim 2 wherein B is selected from the group consisting of Gly-Gly, (\square -N)Lys, Pro-Pro-Xaa-Pro-Pro, -NHCH(X)CH₂SCH₂CO-, -NHCH(X)CH₂SCH₂CO(\square -N)Lys-, -NHCH(X)CH₂S-succinimidyl(\square -N)Lys-, and -NHCH(X)CH₂S-(succinimidyl)-.
- 10 5. A peptide immunogen according to Claim 4 wherein B is Gly-Gly.
6. A peptide immunogen according to Claim 4 wherein B is (\square -N)Lys.
- 15 7. A peptide immunogen according to Claim 1, 2, 3, 4, 5, or 6 wherein the the Target Antigen site is the *Plasmodium falciparum* repeating antigen:
(Asn-Ala-Asn-Pro)_p
(SEQ ID NO:103)
- 20 8. A peptide immunogen according to Claim 7 wherein p=4.
9. A peptide immunogen according to Claim 7 selected from
25 the group consisting of SEQ ID NOS: 104, 105.
10. A peptide immunogen according to Claim 1, 2, 3, 4, 5, or 6 wherein the the Target Antigen site is selected from the group consisting of SEQ ID NO: 106, 107, 108, and 109,
30 an epitope of CETP.
11. A peptide immunogen according to Claim 10 selected from the group consisting of SEQ ID NOS:110 - 119.

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12. A peptide immunogen according to Claim 1, 2, 3, 4, 5, or 6 wherein the the Target Antigen site is selected from the group consisting of SEQ ID NOS: 125, 131, 132, 133, 134, and 135, an epitope of HIV.

5

13. A peptide immunogen according to Claim 12 selected form the group consisting of SEQ ID NOS:126 - 129, 136-151.

14. A peptide immunogen according to Claim 13 selected form the group consisting of SEQ ID NOS:148 - 151.

10

15. A method for producing a peptide immunogen by covalently linking a T helper cell epitope to a target antigenic site selected from the group consisting of B cell epitopes of an antigen and a peptide hapten.

15

16. A method for producing a peptide immunogen according to Claim 15 further linking the covalently linked T helper cell epitope and target antigenic site to an immunostimulatory sequence.

20

17. A method for producing a peptide immunogen according to Claim 16 wherein the immunostimulatory sequence is SEQ ID NO:78.

25

18. A method for producing a peptide immunogen according to Claim 17 wherein B is selected from the group consisting of Gly-Gly, (\square -N)Lys, Pro-Pro-Xaa-Pro-Pro, -NHCH(X)CH₂SCH₂CO-, -NHCH(X)CH₂SCH₂CO(\square -N)Lys-, -NHCH(X)CH₂S-succinimidyl(\square -N)Lys-, and -NHCH(X)CH₂S-(succinimidyl)-.

30

19. A method for producing a peptide immunogen according to Claim 18 wherein B is Gly-Gly.

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20. A method for producing a peptide immunogen according to Claim 19 wherein B is (\square -N)Lys.
- 5 21. A method of inducing T helper cell response by employing a peptide immunogen of claim 1.
22. A method of inducing T helper cell response by employing a peptide immunogen of of claim 2.
- 10 23. A method of inducing T helper cell response by employing a peptide immunogen of of claim 3.
24. A method of inducing T helper cell response by employing a peptide immunogen of of claim 4.
- 15 25. A method of inducing T helper cell response by employing a peptide immunogen of of claim 5.
- 20 26. A method of inducing T helper cell response by employing a peptide immunogen of of claim 6.
27. A method of inducing T helper cell response by employing a peptide immunogen of of claim 7.
- 25 28. A method of inducing T helper cell response by employing a peptide immunogen of of claim 8.
29. A method of inducing T helper cell response by employing a peptide immunogen of of claim 9.
- 30 30. A method of inducing T helper cell response by employing a peptide immunogen of of claim 10.
- 35 31. A method of inducing T helper cell response by employing a peptide immunogen of of claim 11.

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32. A method of inducing T helper cell response by employing a peptide immunogen of of claim 12.
- 5 33. A method of inducing T helper cell response by employing a peptide immunogen of of claim 13.

COMBINED DECLARATION AND POWER OF ATTORNEY FOR
ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL,
DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

Chang Yi Wang

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ARTIFICIAL T HELPER CELL EPITOPES AS IMMUNE STIMULATORS FOR SYNTHETIC PEPTIDE IMMUNOGENS

the specification of which

- a. ☒ is attached hereto
- b. ☐ was filed on _____ as application Serial No. _____ and was amended on _____ (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

- c. ☒ was described and claimed in International Application No. PCT/US99/13975 filed on June 21, 1999 _____ and as amended on _____. (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby specify the following as the correspondence address to which all communications about this application are to be directed:

SEND CORRESPONDENCE TO: MORGAN & FINNEGAN, L.L.P.

345 Park Avenue
New York, N.Y. 10154

DIRECT TELEPHONE CALLS TO: Maria C. H. Lin, Esq.

(212) 758-4800

☐ I hereby claim foreign priority benefits under Title 35, United States Code § 119 (a)-(d) or under § 365(b) of any foreign application(s) for patent or inventor's certificate or under § 365(a) of any PCT international application(s) designating at least one country other than the U.S. listed below and also have

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or Imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(35) I hereby appoint the following attorneys and/or agents with full power of substitution and revocation, to prosecute this application, to receive the patent, and to transact all business in the Patent and Trademark Office connected therewith: John A. Diaz (Reg. No. 19,550), John C. Vassil (Reg. No. 19,098), Alfred P. Ewert (Reg. No. 19,887), David H. Pfeffer (Reg. No. 19,825), Harry C. Marcus (Reg. No. 22,390), Robert E. Paulson (Reg. No. 21,046), Stephen R. Smith (Reg. No. 22,615), Kurt E. Richter (Reg. No. 24,052), J. Robert Dailey (Reg. No. 27,434), Eugene Moroz (Reg. No. 25,237), John F. Sweeney (Reg. No. 27,471), Arnold I. Rady (Reg. No. 26,601), Christopher A. Hughes (Reg. No. 26,914), William S. Feiler (Reg. No. 26,728), Joseph A. Calvaruso (Reg. No. 28,287), James W. Gould (Reg. No. 28,859), Richard C. Komson (Reg. No. 27,913), Israel Blum (Reg. No. 26,710), Bartholomew Verdirame (Reg. No. 28,483), Maria C.H. Lin (reg. No. 29,323), Joseph A. DeGirolamo (Reg. No. 28,595), Michael P. Dougherty (Reg. No. 32,730), Seth J. Atlas (Reg. No. 32,454), Andrew M. Riddles (Reg. No. 31,657), Bruce D. DeRenzi (Reg. No. 33,676), Michael M. Murray (Reg. No. 32,537), Mark J. Abate (Reg. No. 32,527), Alfred L. Haffner, Jr. (Reg. No. 18,919), Harold Haidt (Reg. No. 17,509), John T. Gallagher (Reg. No. 35,516), Steven F. Meyer (Reg. No. 35,613) and Kenneth H. Sonnenfeld (Reg. No. 33,285) of Morgan & Finnegan, L.L.P. whose address is: 345 Park Avenue, New York, New York, 10154; and Edward A. Pennington (Reg. No. 32,588), Michael S. Marcus (Reg. No. 31,727) and John E. Hoel (Reg. No. 26,279) of Morgan & Finnegan, L.L.P., whose address is 1775 Eye Street, Suite 400, Washington, D.C. 20006.

[] I hereby authorize the U.S. attorneys and/or agents named hereinabove to accept and follow instructions from _____ as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and/or agents and me. In the event of a change in the person(s) from whom instructions may be taken I will so notify the U.S. attorneys and/or agents named hereinabove.

Full name of sole or first inventor Chang Yi Wang

Inventor's signature* [Signature] date _____

Residence 47 Snake Hill Road, Cold Spring Harbor, New York 11724 NY

Citizenship U.S.A

Post Office Address Same As Above

Full name of second joint inventor, if any _____

Inventor's signature* _____ date _____

Residence _____

Citizenship _____

Post Office Address _____

identified below such foreign application(s) for patent or inventor's certificate or such PCT international application(s) filed by me on the same subject matter having a filing date within twelve (12) months before that of the application on which priority is claimed:

☐ The attached 35 U.S.C. § 119 claim for priority for the application(s) listed below forms a part of this declaration.

<u>Country/PCT</u>	<u>Application Number</u>	<u>Date of filing (day, month, yr)</u>	<u>Date of issue (day, month, yr)</u>	<u>Priority Claimed</u>
PCT	PCT/US99/13975	June 21, 1999		<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
				<input type="checkbox"/> YES <input type="checkbox"/> NO

☐ I hereby claim the benefit under 35 U.S.C. § 119(e) of any U.S. provisional application(s) listed below.

Provisional Application No.

Date of filing (day, month, yr)

**ADDITIONAL STATEMENTS FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART
OR PCT INTERNATIONAL APPLICATION(S DESIGNATING THE U.S.)**

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or under § 365(c) of any PCT international application(s) designating the U.S. listed below.

<u>09/100,412</u>	<u>June 20, 1998</u>	<u>Abandoned</u>
US/PCT Application Serial No.	Filing Date,	Status (patented, pending, abandoned)/ U.S. application no. assigned (For PCT)
<u> </u>	<u> </u>	<u> </u>
US/PCT Application Serial No.	Filing Date,	Status (patented, pending, abandoned)/ U.S. application no. assigned (For PCT)

☒ In this continuation-in-part application, insofar as the subject matter of any of the claims of this application is not disclosed in the above listed prior United States or PCT international application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

[] ATTACHED IS ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY THIRD AND SUBSEQUENT INVENTORS FORM.

* Before signing this declaration, each person signing must:

1. Review the declaration and verify the correctness of all information therein; and
2. Review the specification and the claims, including any amendments made to the claims.

After the declaration is signed, the specification and claims are not to be altered.

To the inventor(s):

The following are cited in or pertinent to the declaration attached to the accompanying application:

Title 37, Code of Federal Regulation, §1.56

Duty to disclose information material to patentability

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

Title 35, U.S. Code § 101

Inventions patentable

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Title 35 U.S. Code § 102

Conditions for patentability; novelty and loss of right to patent

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent,

(b) the invention was patented or described in a printed publication in this or foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States, or

(c) he has abandoned the invention, or

(d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months before the filing of the application in the United States, or

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or

(f) he did not himself invent the subject matter sought to be patented, or

(g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other ...

Title 35, U.S. Code § 103

Conditions for patentability; non-obvious subject matter

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Title 35, U.S. Code § 112 (in part)

Specification

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Title 35, U.S. Code, § 119

Benefit of earlier filing date in foreign country; right of priority

An application for patent for an invention filed in this country by any person who has, or whose legal representatives or assigns have, previously regularly filed an application for a patent for the same invention in a foreign country which affords similar privileges in the case of applications filed in the United States or to citizens of the United States, shall have the same effect as the same application would have if filed in this country on the date on which the application for patent for the same invention was first filed in such foreign country, if the application in this country is filed within twelve months from the earliest date on which such foreign application was filed; but no patent shall be granted on any application for patent for an invention which had been patented or described in a printed publication in any country more than one year before the date of the actual filing of the application in this country, or which had been in public use or on sale in this country more than one year prior to such filing.

Title 35, U.S. Code, § 120

Benefit or earlier filing date in the United States

An application for patent for an invention disclosed in the manner provided by the first paragraph of section 112 of this title in an application previously filed in the United States, or as provided by section 363 of this title, which is filed by an inventor or inventors named in the previously filed application shall have the same effect, as to such invention, as though filed on the date of the prior application, if filed before the patenting or abandonment of or termination of proceedings on the first application or an application similarly entitled to the benefit of the filing date of the first application and if it contains or is amended to contain a specific reference to the earlier filed application.

Please read carefully before signing the Declaration attached to the accompanying Application.

If you have any questions, please contact Morgan & Finnegan, L.L.P.

FORM: COMB-DEC.NY
Rev. 1/22/98